FACTORS AFFECTING SEVERITY OF INJURY IN ALLERGIC RENAL DISEASE

BY

ROAL VAN ZYL-SMIT

MBBCh FCP(SA) MRCP(UK)

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ABSTRACT

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Experimental studies of nephrotoxic nephritis in animals have demonstrated many of the underlying pathogenetic mechanisms involved in human glomerulonephritis.

The first part of this thesis reviews the literature with regard to the factors and mechanisms responsible for the causation of glomerulonephritis and those factors which determine severity and allow for its persistence.

In order to study these mechanisms, a reproducible model of nephrotoxic nephritis (NTN) in rabbits was established. This disease is produced by the intravenous injection of nephrotoxic serum, raised in sheep, containing antibodies to rabbit glomerular basement membrane.

In a second group of experiments, the induction of an acute phase response in rabbits by the subcutaneous injection of a local irritant was studied. It was characterised by changes in C-reactive protein (CRP), the third component of complement (C3), fibrinogen and polymorphonuclear leucocytes (PMN's), however renal function and renal histology were not affected in any way.

When the so called heterologous phase of NTN in rabbits was induced during maximal acute phase stimulation, it did not result in enhancement of injury when compared to unstimulated rabbits. A similar situation was found during

the autologous phase, where a superimposed acute phase stimulus again did not cause enhancement of injury.

Despite the fact that CRP has been shown to localize in injured tissue under certain circumstances, it did not fix in the kidneys of rabbits with induced NTN, nor in human kidneys affected by a variety of glomerulonephritides.

The reasons for the variation in severity of disease during the autologous phase were analysed. Rabbits which developed severe glomerulonephritis during the autologous phase of NTN characteristically produced high titres of rabbit anti-sheep antibody early in the disease when compared to those which did not develop injury, even though they might ultimately have developed the same titre of antibodies. One of the major determinants of injury appears to have been the rate at which antibody bound to the glomerular basement membrane (GBM). Studies during the heterologous phase confirmed this impression when it was shown that nephrotoxic globulin (NTG) given slowly, produced less injury than when the same amount was infused fast. This observation also suggested that the effector function of immunoglobulin decays much more rapidly than was previously thought.

Finally, a mechanism of "protection" from injury by circulating antibody was shown by the phenomenon of "saturation" of available antibody binding sites on the

glomerular basement membrane which appeared to limit the deposition of further circulating antibody thus preventing further damage.

These studies have advanced the understanding of human glomerulonephritis by illustrating how variation in immune responsiveness may contribute to the development of disease. They suggest that something inherent in infections, other than merely an acute phase response, is responsible for infection induced relapse in certain cases of allergic renal disease. By demonstrating a mechanism of "protection" from the effect of circulating antibodies, these studies suggest that therapy directed towards blocking of available antigenic sites on the human glomerular basement membrane, may become a therapeutic reality.

STATEMENT OF CANDIDATE

I declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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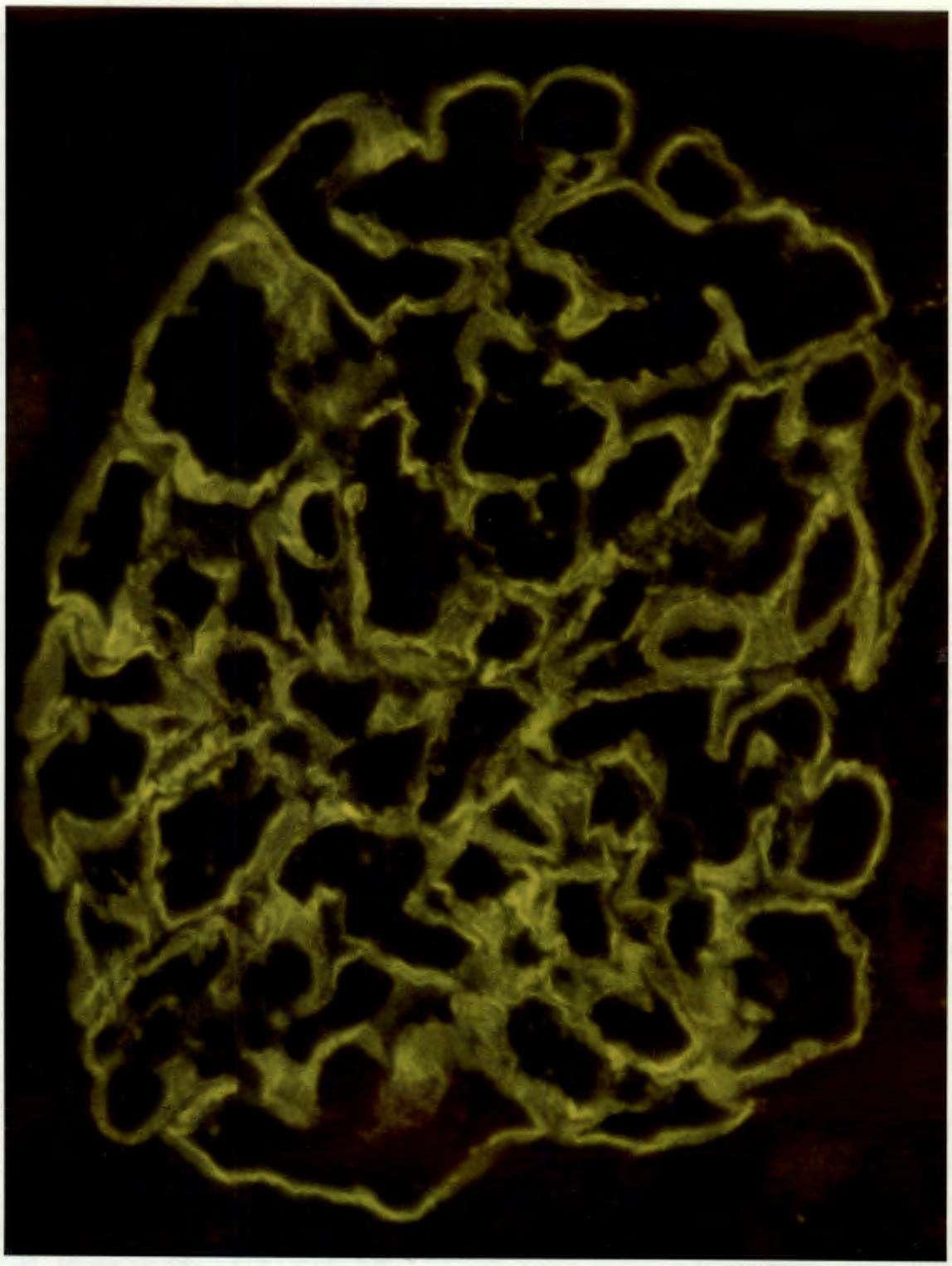
INTRODUCTION

NEPHROTOXIC NEPHRITIS IN RABBITS

A RABBIT GLOMERULUS



Binding of antibody to rabbit glomerular basement membrane demonstrated by a direct immunofluorescent technique.

INTRODUCTION

Allergic processes both humoral and cellular and their mediator systems including complement, clotting factors, kinins, polymorphs and macrophages participate in the pathogenesis of most cases of human glomerulo- and tubulo-interstitial nephritis. "Non-immune mechanisms" such as hypertension, glomerular fluid dynamics, carbohydrate metabolism, and even proteinuria itself may either potentiate injury or allow for its persistence after cessation of the initial insult.

In some varieties such as post-streptococcal glomerulonephritis the initiating causes and immunopathologic events leading to injury have been extensively studied and are fairly well understood.

With most varieties, however, very little is known about initiating factors, exact mechanisms whereby damage is caused and factors which allow for persistence of injury. Unfortunately even less is known about effective therapy for most of these disorders.

Animal models, whilst illustrating some basic immunopathogenetic mechanisms, are most often not true replicas of human glomerulonephritis and information gained is not easily transferred, even between different animal models.

One of the most intriguing aspects of all types of glomerulonephritis is the question why only some individuals get the disease and coupled with this, why there is so much variation in the severity and persistence of injury once the disease process has begun.

Anti-glomerular basement membrane antibody (anti-GBM) disease in humans is generally a rapidly progressive and often fatal disease yet, some patients remain well over prolonged periods of time, despite the presence of high titres of anti-GBM antibody in their circulation and deposited in their kidneys.

This phenomenon is well illustrated by two cases described by Bailey (1981) - both presenting with haemoptysis and haematuria and both having high titres of anti-GBM anti body in their circulation.

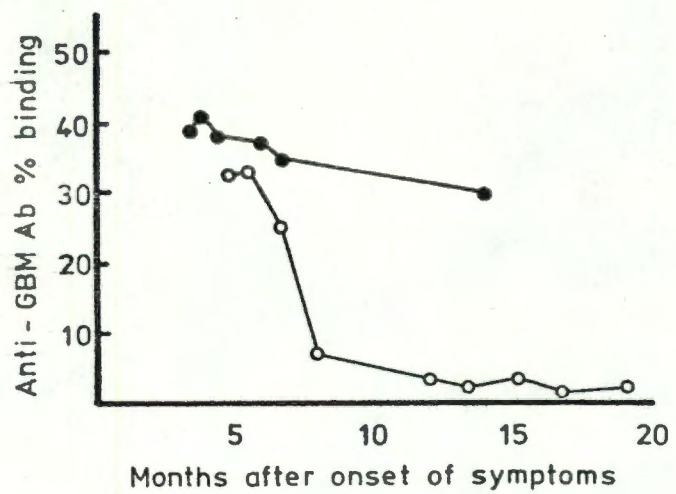


Fig.1 Anti GBM antibody levels. (Months after onset of symptoms.) Normal levels = < 1% binding. [After Bailey]

Despite the fact that these patients received no treatment and high levels of antibody persisted, their renal function did not deteriorate over a 6 month follow up period.

Conversely, the clinical observation has frequently been made that enhanced injury in various forms of glomerulonephritis follows apparently unrelated stimuli such as infections, trauma and severe exercise. An example of this phenomenon is shown in Figure 2 in a patient with anti-GBM antibody disease, described by Rees et al (1978).

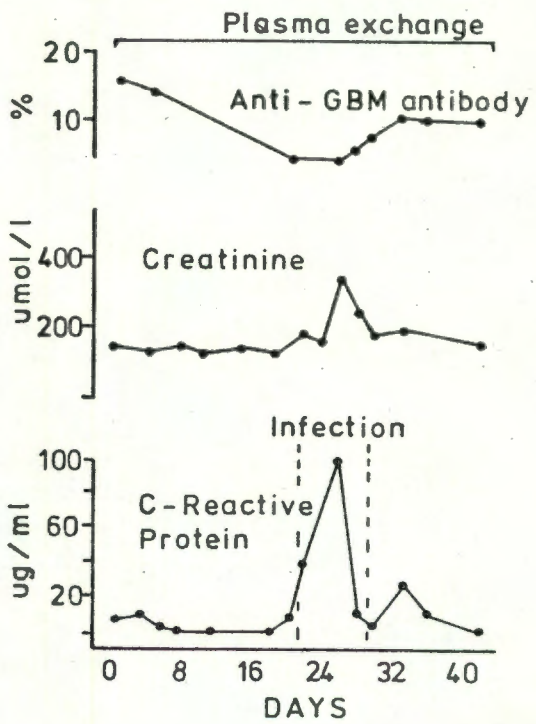


Fig.2 Relapse in anti-GBM antibody mediated disease

The patient had gone into remission on treatment with plasma exchange and immunosuppressives, but relapsed during

an intercurrent infection in a forearm shunt. (used for plasma exchange). Haematuria and haemoptysis occured and his renal function deteriorated.

The serum C-reactive protein (CRP) level, measured by the routine CRP assay which I had developed at Hammersmith Hospital shortly before this patient was studied, rose dramatically during the infection, as expected in an "acute phase response". On treatment of the infection everything, including the "acute phase response", resolved. Of note was the fact that anti-GBM antibody levels remained constant during this exacerbation of disease activity, and rose only slightly during the recovery phase.

The first half of this thesis addresses itself to certain aspects of this phenomenon by examining the effect of an acute phase stimulus (without infection) on the severity and outcome of the two phases, the so-called heterologous and autologous phases, of nephrotoxic nephritis (NTN).

This experimental model of glomerulonephritis in rabbits was first described by Masugi in 1933.

V.

Über das Wesen der spezifischen Veränderungen der Niere und der Leber durch das Nephrotoxin bzw. das Hepatotoxin.

Zugleich ein Beitrag zur Pathogenese der Glomerulonephritis und der eklamptischen Lebererkrankung.

Von

Prof. Dr. Matazo Masugi.

Aus dem Pathologischen Institut der med. Universität zu Chiba, Japan.

(Eingegangen am 6. Juli 1932.)

This model was chosen because of the similarities between it and human anti-GBM mediated diseases. During the "heterologous" phase of NTN, sheep anti-rabbit GBM is infused, whilst during the autologous phase, autologous rabbit antibody is directed towards planted antigens (sheep antibody) on its own GBM.

Considerable attention was paid to the possible role of C-reactive protein (CRP) because of the tremendous rise in its concentration during the acute phase response and its known capacity to interact with many allergic and inflammatory reactions. In addition, a relationship has been suggested between the capacity of subjects to produce antibodies and the magnitude of their CRP response. (Wood 1953)

The second part of this thesis examines some of the

mechanisms responsible for the variation in severity of nephrotoxic nephritis (NTN) in a large group of rabbits, all having been exposed to the same initiating factors but manifesting great differences in severity of disease. This phenomenon has a human parallel as it is well known that when exposed to the same environmental factors, only some persons develop glomerulonephritis, whilst others appear to be "immune".

Finally, a model both in vivo and in vitro was established to illustrate some of the possible mechanisms involved in preventing glomerulonepritis, despite the presence of circulating anti-GBM antibody, a situation not infrequently found in patients with anti-GBM antibody disease in remission.

PART I

FACTORS INVOLVED IN THE CAUSATION OF GLOMERULONEPHRITIS

PART I

FACTORS INVOLVED IN THE CAUSATION OF GLOMERULONEPHRITIS

I.1.0 GENETIC FACTORS

I.1.1 Major Histocompatibility Complex (MHC) associations

In experimental nephritis a genetic dependency upon the expression of a variety of immune responses has been well described, for example, " immune complex" glomerulonephritis in rats (Stenglein 1975, Sugisaki 1973) and "anti-glomerular basement membrane (anti-GBM) antibody" disease in rats (Druet 1977).

Hybrid breeding experiments with Lewis rats (lacking a nephritogenic antigen) and Brown Norway rats (carrying a nephritogenic antigen) which have a strain susceptibility to develop auto antibodies to GBM following mercuric chloride exposure, have demonstrated a genetic factor linked to the MHC contained in the H-1n haplotype (Druet 1977) and a factor which interacts with a gene outside of the MHC (Sapin 1981).

Similarly in (NZB x NZW) F1 hybrid mice manifesting with an immune complex nephritis similar to human systemic lupus erythematosus (SLE), the control of abnormal auto antibody synthesis resides outside of H2 (Raveche 1978) whilst susceptibility of certain strains of guinea pigs to develop

an abnormal antibody to tubular basement membrane (TBM) is controlled by a factor in the MHC (Hyman 1976).

By contrast it is variation in the antigenic structure of rat TBM which determines strain variation in anti-TBM antibody disease induced by immunization with heterologous TBM and not their capacity to produce antibodies (Kreiger 1981).

In humans the first example of the link between certain kinds of glomerulonephritis and the MHC was noted in IgA nephropathy (Noel 1978) and Henoch Schoenlein Purpura (Nyulassi 1977). Both these diseases are characterized by the deposition of IgA as the predominant immunoglobulin in the mesangium and are associated with BW35 (Noel 1978) and possibly DR4 (Fauchet 1980). (Both these associations still await final confirmation.)

In the United Kingdom membranous nephropathy is associated with the haplotype HLA DR3 B18 BfF1. It also appears that patients bearing this haplotype are less likely to go into remission than those missing some elements of this haplotype (Cairns 1980).

The association of anti-GBM mediated Goodpasture's disease with HLA DRW2 was first shown by Rees (1978). Subsequent work seems to indicate that DRW2 predisposes to disease whilst the presence of HLA B7 may have an adverse effect on the course of the disease (Rees 1981).

Minimal change nephropahy is associated with HLA B12 (Thomson 1976, Trompeter 1980) and HLA DR7 which is known to be in linkage disequilibrium with B12 (Alfiler 1980).

Other examples of genetic predisposition to glomerulonephritis include (i) The association of mesangiocapillary glomerulonephritis with a specific B - cell allo-antigen (Friend 1977), (ii) (SLE) and the B lymphocyte alloantigens HLA DRW2 and HLA DRW3 (Reinertsen 1978) and (iii) the glomerulonephritis found in association with autoimmune disease and C2 deficiency (Glass 1976).

Many mechanisms for this apparent genetically determined predisposition to renal disease have been suggested.

I.1.2 Variation in antibody responses

Soothill (1971) and subsequently Devey and Steward (1980) showed that nephritis prone strains of inbred mice produce lower avidity antibodies to foreign proteins when compared with nephritis-resistant mice. This may be linked with the observation that chronic nephritis is more often found in association with the presence of non-precipitating than with precipitating antibodies (Dixon 1961, Pincus 1968).

This variation of immune responsiveness at the level of antigen recognition has been shown in the mouse to be

regulated by immune response genes (Benaceraff 1972, 1981), whilst antibody levels and antibody affinity are under independent genetic control (Katz 1976, Steward 1979).

In human nephritis it has been noted in patients with SLE that those with non-precipitating antibodies tended to develop a picture of a membranous nephropathy whilst those with precipitating antibodies more often had either no nephritis or a proliferative picture (Friend 1977). Others have noted an association between high avidity antibodies and the presence and severity of nephritis in SLE (Leon 1977, Winfield 1977). Furthermore, the sub-class of IgG involved also had an effect: high titres of complement fixing antibodies (predominantly IgG2) were found more often in patients with severe renal involvement (Schur 1968).

There is little evidence that complete IgG deficiency is associated with an increased tendency to develop nephritis (Rashid 1981). IgA deficiency is associated with a number of immunological disorders such as SLE but again no increased incidence of nephritis is noted (Yewdall 1982).

The exact mechanisms whereby variation in antibody responses may affect the development of nephritis will be discussed in part V.

I.1.3 Immunodeficiency

B cell, T cell and immunoglobulin deficiencies are frequently associated with conditions such as recurrent infections. A deficiency of peripheral blood suppressor T lymphocytes has been reported in patients with active SLE and in some forms of idiopathic glomerulonephritis (Morimoto 1980, Chatenoud 1981) .

The most striking example of immunodeficiency associated with renal disease is however, seen with the great variety of complement deficiencies (reviewed by Jerslid 1976, Schur 1977, Schur 1978, Agnello 1978).

The most common complement deficiency, that of C2, is found in approximately 1 in 10000 of the general population and is associated with SLE like syndromes with renal disease and vasculitis (Agnello 1978).

Most of the renal disease occurs in association with the early complement component deficiency states (see review articles) but it has also been described with deficiencies of C5, C8 and C1 esterase inhibitor deficiency (Kohler 1976).

The mechanism whereby complement deficiency predisposes to renal disease is not clear. One possibility is the association with HLA antigens, as C4d, C2d and factor B of the alternative pathway are all coded on the sixth

chromosome (Schur 1978). Strong linkage disequilibrium exists between the loci for C2d and the HLA haplotype A10 B18 (Schur 1978). As the immune response genes are also probably linked to HLA, the possibility exists that C2d may merely be a marker for a deficient immune response gene, which might be responsible for the increased susceptibility to disease.

However, not all the complement components are encoded in the MHC (C3, C1 inhibitor and components of the C1 complex). Therefore it is more likely that the disease susceptibility is linked to complement function rather than a disease susceptibility gene located within the MHC.

Possible mechanisms in relation to defective complement function include: defective clearance of circulating immune complexes (by C2 and C4) (Mannik 1971); defective viral neutralization by the alternative pathway of complement (Daniels 1969); and defective solubilization of deposited immune complexes by the alternative pathway (Czop 1976, Miller 1975). Bartolotti (1978) showed in rabbits with acute serum sickness that decomplementation with cobra venom factor delayed the clearance of complexes from the kidney, presumably also on the basis of defective solubilization. Another mechanism is that defective early complement components fail to stabilize newly formed immune complexes, which allows their precipitation in blood vessels before they can be transported to the reticulo

endothelial systems for phagocytosis and disposal (Shifferli 1981).

A completely different kind of complement deficiency exists in patients with mesangiocapillary glomerulonephritis with or without associated partial lipodystrophy (Sissons 1976). Here low C3 levels may be found often ante-dating the onset of renal disease. The C3 is broken down excessively as a result of stabilization of the alternative pathway convertase C3b Bb by the abnormal "nephritic factor" NeF. This factor is an IgG auto antibody (Scott 1978) which binds to the alternative pathway convertase and blocks the functions of the C3b Bb inactivators factor H (previously known as BlH protein) and factor I (previously known as C3b inactivator).

A similar phenomenon involving the classical pathway C3 convertase has also been described (Halbwachs 1980). This abnormal factor interferes with the function of the classical pathway convertase (C4bC2b) control proteins, C4 binding protein and factor I.

A third mechanism of stabilization of the alternative pathway C3bBb convertase by inhibition of factor H has been described in a patient with cryoglobulinaemia and the subendothelial deposit variety of mesangiocapillary glomerulonephritis (Bartlow 1979).

The exact mechanism whereby these "activators" appear to

predispose to nephritis is uncertain. It is unlikely that they act merely by inducing a state of "complement deficiency" similar to the genetic deficiencies. The classic type of nephritis associated with NeF is the so-called "dense deposit disease" variety of mesangiocapillary glomerulonephritis (and less commonly the variety characterized by the deposition of subendothelial immune complexes). Dense deposit disease is different from all other types of immune complex or antibody mediated nephritis. The deposits may represent the accumulation of complement breakdown products, with the continuing complement activation possibly disrupting the basement membrane chemistry - none of these hypotheses are however as yet proven.

I.2.0 PRIMARY MECHANISMS OF INJURY IN GLOMERULONEPHRITIS

I.2.1 Introduction

Allergic mechanisms causing renal injury are very complex and involve predominantly antibodies and their effector systems which may be summarised as follows.

(a) Local antibody reactions

(i) Directed towards tissue specific antigens such as basement membrane antigens (GBM, TBM) and non-basement membrane antigens in glomeruli and tubules.

(ii) Directed towards "planted" antigens (immunoglobulins, mesangial material, lectins, DNA, etc.)

(b) Circulating immune complex reactions

(i) Exogenous and/or endogenous antigens with complexes localized in glomeruli or tubulo interstitial areas.

(ii) Continuing reactivity of localized immune complexes with circulating antigen or antibody.

The role of cellular immune mechanisms are far less clear, whilst a large group of diseases exist where the mechanisms responsible for the disease are entirely unknown.

I.2.2 Local antibody reactions

The nephrotoxicity of heterologous anti-kidney antiserum was first noted by Lindemann in 1900. The antigen to which this antiserum was directed was subsequently shown to be located in the glomerular basement membrane (Krakower and Greenspon 1951). Subsequently the pathogenicity of anti glomerular basement membrane (GBM) antibodies has been demonstrated by many workers (Unanue 1967, Wilson 1976) and tests for their detection in the circulation have been perfected (Wilson 1974, Lockwood 1979). Similarly more rational therapy based on the removal of pathogenic anti-GBM antibody by plasma exchange was begun (Lockwood 1976).

The antigens involved in experimental forms of anti-GBM antibody disease have been shown to lie within the non-collagenous glycoprotein of the GBM (Marquardt 1973) with molecular weights of 53 000 and 27 000 (Holdsworth 1979). Subsequent studies by Fish (1979) have shown that serum taken from patients with anti-GBM antibody disease binds specifically to antigens distributed along the inner (endothelial side) lamina of the basement membrane whereas in certain experimental models, antigens may be recognized by nephrotoxic antibody in both the inner and outer layers of the GBM (Fish 1979).

(i)Experimental anti-basement membrane antibody nephritis

When heterologous anti-basement membrane antibody is injected into an experimental animal, injury occurs in two phases. The heterologous phase occurs within a few hours provided that sufficient nephrotoxic antibody has bound to the kidney. Threshold levels of antibody binding to kidney required to produce proteinuria are: rat 75 ug/g kidney, rabbit 15 ug/g kidney and 5 ug/g kidney in sheep (Unanue and Dixon 1967). The species of animal in which the heterologous antiserum was raised also affects its "nephrotoxic" properties and the dose needed to produce proteinuria. Proteinuria is maximal between 24 to 48 hours and then settles spontaneously.

The autologous phase follows after a delay of some days (4 - 8) when the host responds by making an autologous antibody to the foreign or "planted" antigen. Injury follows and tends to be progressive with not only proteinuria but also varying degrees of glomerular damage and renal failure causing the death of some animals. Others, however, gradually recover over a period of weeks. This reaction may occur when as little as 2 ug ab/g kidney is bound to the basement membrane (Unanue 1965). Considerable augmentation of this phase may be induced by pre-immunization of the animal to the foreign immunoglobulin, producing the so called "accelerated" phase of nephrotoxic nephritis.

Many other experimental models of anti-GBM nephritis exist, such as nephritis in sheep (Steblay 1962), anti-tubular basement membrane nephritis (Wilson 1981) and mercuric chloride induced anti-TBM nephritis (Lehman 1974).

(ii) Human anti glomerular basement membrane (GBM) antibody diseases

Originally Goodpasture's disease and anti-GBM antibody disease were considered synonymous. It has, however, become apparent that a whole spectrum of conditions is associated with circulating antibodies to renal structures. These range from severe progressive crescentic nephritis with associated pulmonary haemorrhage (the full blown picture of Goodpasture's syndrome) to cases presenting with mild haemoptysis and no renal disease whatever (Wilson 1973).

Anti-GBM antibodies may in addition cross-react with the renal tubular basement membrane in roughly 70% of cases (Lehman 1975), with the basement membrane of the choroid plexus (Wilson 1981) and even with the basement membrane of the intestine (Wilson 1981). Persistence of circulating antibodies after total renal failure may cause recurrence of the disease with renal transplantation (Wilson 1973). Other problems following renal transplantation occur when humans lacking certain nephritogenic antigens receive a normal kidney containing these antigens. This evokes a response against "normal" GBM antigens and rejection of the

kidney may occur (Wilson 1974, McCoy 1976).

(iii) Relationship of antibody level to severity of disease

It is clear that many factors other than levels of antibody interact to produce disease. This is evidenced by the fact that often very high levels of anti-GBM antibody exist without overt disease (Bailey 1981, Simpson 1982). This is particularly so in cases with associated pulmonary disease (Wilson 1978).

Similarly, relapses of glomerulonephritis as well as pulmonary disease (haemoptysis) may occur without demonstrable rises in the level of circulating antibody (Rees 1977, Johnson 1978). It appears that some additional factors such as infections and possibly physiologic changes may be involved - these issues are discussed in detail in Part I.5.0

(iv) Glomerulonephritis related to non-GBM capillary wall antigens

"Heymann" nephritis (1959) is induced in rats by repeated immunization with rat kidney homogenates. A membranous type of glomerulonephritis results which initially was thought to be the result of deposition of circulating immune complexes. However, transfer of disease with passive administration of sera from immunized animals

(Sugisaki 1973, Barabas 1974) has suggested that anti-tubular basement membrane antigens react directly with non-basement membrane antigens, possibly with discrete areas at the bases of epithelial foot processes (Neale 1979). Couser (1978) using an isolated perfused kidney model also demonstrated a discontinuous distribution of an antigen cross-reacting with tubular antigen distributed along the outer aspect of the glomerular basement membrane. (beneath the epithelial cells)

(v)Glomerulonephritis due to trapped or "planted" antigens

(a) Experimental models

Many types of foreign material may be planted on the glomerular basement membrane such as heterologous antibody, which is deposited on the glomerular basement membrane during the first phase of experimental nephrotoxic nephritis (This thesis, part II). During the so-called "autologous" phase, autologous antibody produced in response to the foreign protein (heterologous antibody) which was injected, reacts with the "planted" heterologous antibody on the basement membrane.

Other "planted" antigens include heat aggregated human immunoglobulin which, when injected into rabbits, localizes in the mesangium and will react with passively injected anti human IgG producing a type of glomerulonephritis

(Mauer 1973).

Sequential injection of heparin and protamine also results in subepithelial aggregates (Sharon 1977) whilst a similar phenomenon has been described with DNA (Izui 1976), BSA (Fleuren 1980), and the lectin, concanavalin A (Golbus 1979).

The passage of antigen across the poly-anion rich basement membrane is facilitated by a positive charge. Therefore injecting cationic BSA into rabbits repeatedly leads to a membranous nephropathy (Border 1982) whilst animals given neutral or anionic BSA only rarely develop nephritis (Germuth 1973, Border 1982). This reaction may also be blocked by injecting protamine as a competing cation (Adler 1981).

Substances in the circulation which might bind to the antigen and cause persistence of the glomerulonephritis include, besides antibody, complement, nephritic factor, rheumatoid factor, anti-idiotypic antibodies and immuno conglutinins (Wilson 1981, Agnello 1983, Schreiber 1979).

(b) Possible relevance to human disease

The model of lectin binding to the GBM (Golbus 1979) leads to speculation that many infective agents in man, having lectin-like properties, may localize in capillary walls and act as planted antigens elicting a localized immune

reaction and glomerulonephritis.

At present there is no definite evidence that a similar mechanism of in situ immune complex formation plays an important role in the pathogenesis of human glomerulonephritis. There are however individual case reports which suggest that this mechanism may operate occasionally, for example,in idiopathic membranous nephropathy where renal tubular antigens deposit in the classical subepithelial situation (Douglas 1981).

In patients with SLE, DNA has been shown to have a marked affinity for collagen and therefore by binding to the GBM may act as a "planted antigen" capable of reacting with anti-DNA antibody to form local complexes (Izui 1976).

I.2.3 Circulating immune complex reactions

The local immune reaction which develops in the kidney after localization of circulating immune complexes appears to be responsible for about 80% of human glomerulonephritides and an undefined number of cases of tubulo interstitial nephritis. Many of these cases are associated with systemic diseases characterized by the presence of circulating immune complexes, e.g. SLE, whilst others, despite the presence of circulating immune complexes, eg post streptococcal GN, seem to affect the kidney only. In other cases there is clear evidence of immune complex

localization in the kidneys with only minimal detectable circulating complexes.

Multiple antigen-antibody systems may combine or operate in sequence to cause injury and the apparent amount of immune complex needed to produce this injury seems to vary widely (Review Cochrane 1973, Wilson 1981).

Recently, however, the true role of immune complexes as a primary cause of glomerulonephritis has been questioned (Couser 1980).

It is difficult to extrapolate from animal experiments to humans as, for example, platelet function in humans and rabbits varies so widely that the well defined role of platelet vasoactive amines in promoting the localization of circulating immune complexes in rabbits has been questioned in humans as an important mechanism. An exception possibly exists in some cases of SLE (Egido 1980).

Furthermore, high levels of circulating complexes may be found in patients with no renal disease, e.g. rheumatoid arthritis (Gupta 1979) whilst in patients with glomerulonephritis there is very seldom any correlation between the presence or level of immune complexes (IC's)and disease activity (Border 1979).

Finally, it is extremely difficult to decide whether ICs are

in fact pathogenic as they may occur in the circulation and glomerular mesangium in infectious diseases without any evidence of nephritis whatever (Sitprija 1974). Renal localization may be one of the normal mechanisms of elimination of complexes, as is mononuclear phagocyte function in general.

Impairment of mononuclear phagocyte function in patients with circulating immune complexes has been reported by Lockwood (1979) and Lawley (1980). This acquired deficiency, possibly due to the effect of the complexes themselves may allow the persistence of potentially toxic complexes in the circulation for longer periods, which may potentiate injury.

The antigens involved in circulating immune complex reactions may be classified into two broad catagories. Those arising from endogenous sources, and those arising from exogenous sources.

1. Diseases due to exogenous antigens

The following table lists some of the exogenous antigens known to be associated with immune complex glomerulonephritis.

IMMUNE COMPLEX GLOMERULONEPHRITIS	
A.EXOGENOUS ANTIGENS	DISEASE
(i) Bacterial: Streptococcus,staphylococcus, T.pallidum, M.leprae, S.typhi, enterococcus	Post-streptococcal GN, infected AV-shunts, pneumonia, endocarditis, secondary syphilis, leprosy, typhoid.
(ii)Viral: Hepatitis B, measels, mumps, Ebstein-Barr virus, oncorna-virus.	Hepatitis, subacute sclerosing pan-encephalitis, mumps, infectious mononucleosis, Burkitts' lymphoma leukaemia.
(iii)Parasites: P.malariae, P.falciparum, T.gondii, S.mansoni.	Malaria, toxoplasmosis, schistosomiasis, kala azar.
(iv)Drugs: Serum,innoculations,drugs, heavy metals.	Serum sickness, membranous nephropathy, (eg gold induced nephrotic syndrome)
B.ENDOGENOUS ANTIGENS	DISEASE
(i) Nuclear antigens	Systemic lupus erythematosus
(ii)Renal tubular antigens	Membranous GN, sickle cell anaemia renal carcinoma, reflux nephropathy(?)
(iii)Tumour antigens (eg carcino-embryonic antigen)	Neoplasia
(iv)Immunoglobulin	Cryoglobulinaemia
(v) Thyroglobulin	Thyroiditis + nephritis.
C.UNKNOWN ANTIGENS	DISEASES
	Mesangio capillary glomerulo - nephritis, membranous nephropathy sarcoidosis, polyarteritis nodosa, (some cases) Henoch Schonlein purpura, scleroderma, IgA-IgG nephropathy, IgM nephropathy, some cases of rapidly progressive GN, focal GN,chronic glomerulonephritis lymphoma, Guillain-Barre syndrome

Table I.1

Immune complex glomerulo nephritis

2. Diseases due to endogenous antigens

The classic example of this type of GN is that associated with SLE where the predominant antigen is native DNA. Virtually the entire spectrum of renal pathology is associated with this disease. Other endogenous antigens include thyroglobulin (O'Regan 1976), renal tubular antigens (Naruse 1973), neoplasia (Couser 1974), carcinoembryonic antigen (Costanza 1973), and cryoglobulins (McIntosh 1975).

3. Diseases due to unidentified antigens

In the majority of cases of adult glomerulonephritis thought to be associated with circulating complexes, the antigen to which the antibody has been directed is unknown and hence treatment and prevention of this condition is much more difficult. Possible explanations for this lack of defined antigens include: an unidentified virus may be responsible; some other exogenous antigen in the environment or water; possibly also the original initiating antigen might have disappeared and been replaced by other substances such as autologous antibody, rheumatoid factor, or even anti-idiotype antibodies.

I.2.4 Glomerulonephritis due to disorders of cell-mediated immunity

In 1970 Dixon stated that there was no definite evidence that cell mediated immunity was involved in the production of human glomerulonephritis. However, this picture has been gradually changing. It is now known that cell-mediated immunity may participate as part of the overall immune response in humans (Rocklin 1970, Mahieu 1972). Changes consistent with cell-mediated immunity have been described in several experimental models. Crescent formation in Masugi nephritis (Kondo 1972); mononuclear cells in experimental nephritis (Schreiner 1978); cellular immunity in association with anti-GBM antibodies in experimental nephritis (Lehman 1976); macrophages in crescent formation (Atkins 1976) and mediating capillary injury in experimental serum sickness (Holdsworth 1981). Bhan (1978) showed that cell transfer to recipient animals immunized against anti-GBM antibody, bearing "planted" immune complexes containing BSA, could induce acute nephritis, suggesting a cell-mediated response.

A very exciting observation was made recently by Schreiner (1981) when he noted the presence in glomeruli of a resident population of cells (monocytes) bearing Ia (DR) determinants. These cells reacted specifically with lymphocytes in a MHC-restricted fashion suggesting that no longer should the kidney be considered an "innocent bystander" but rather that it may play a primary role in modulating local

immune reactions.

In humans the renal interstitium appears more frequently involved in cellular reactions. Sensitization to renal basement membrane antigens may be responsible for the mononuclear cell infiltrate seen in patients with anti-GBM nephritis (Rocklin 1970). Anti-TBM antibodies, IgE antibodies or delayed hypersensitivity reactions may play a role in drug induced interstitial nephritis (McCluskey 1978). Finally it appears that cellular immune reactions play a prominent part in transplant rejection, particularly with the finding of Ia (DR) related antigens in the kidney. (Schreiner, 1981)

I.2.5 Glomerulonephritis of uncertian pathogenesis

Some of these diseases are probably mediated via immune complexes, eg. Henoch Schonlein purpura, Wegener's granulomatosis, polyarteritis nodosa, sarcoidosis and multiple sclerosis (Wilson 1976).

In others the pathogenetic mechanisms are far less clear, for example, minimal change disease, diabetes mellitus, hereditary renal disease, coagulopathies such as the haemolytic uraemic syndrome and the renal lesion of pre-eclamptic toxaemia (Kincaid-Smith 1975).

1.3.0 MEDIATORS OF ALLERGIC RENAL INJURY

1.3.1 Introduction

Several mechanisms interact to cause allergic injury of glomeruli and tubules. The best studied are the complement system, polymorphonuclear leucocytes, the mononuclear phagocyte system (MPS), and the coagulation system.

Central to all these reactions are the immunoglobulins which initiate virtually all the reactions that occur in the glomeruli and cause amplification of injury by recruitment of the inflammatory mediators discussed in this section.

1.3.2 Immunoglobulins.

Despite the fact that immunoglobulin molecules play a central role in allergic reactions, little is known at a molecular level about the mechanism by which immunoglobulin molecules mediate effector funtions. Similarly very little is known about the rate of biologic ageing or decay of effector functions of these molecules. This is further investigated in part V of this thesis.

Immunoglobulin effector function

It is thought that virtually all immunoglobulin effector function is localized to the Fc portion of the molecule.

However in certain experimental models it has been possible to define certain types of injury, independant of Fc function.

F(Ab) related effector function

In the model of Guinea Pig nephrotoxic Nephritis (NTN) it has been possible to define a mechanism of injury independant of Fc function (Couser 1977, Simpson 1975). Here marked proteinuria is produced by the binding of bivalent $F(Ab)_2$ fragments of sheep nephrotoxic globulin. Proteinuria occurs, independant of the action of complement, polymorphs, and other inflammatory mediators such as vasoactive amines, kinins, prostaglandins, the coagulation system or substances released by sensitized lymphocytes.

Massive transient proteinuria is induced by the administration of a small dose of nephrotoxic antibody (resulting in the binding of 15 ug Ab/kidney) (Simpson 1975).

Two mechanisms may contribute to this proteinuria.

(a) Transient decreases in renal plasma flow, nephron filtration rate and urine flow have all been documented in the early phase of NTN (Blantz 1976). Ryan, Karnovsky and Hein demonstrated increased penetration of albumin, IgG and catalase through the GBM in conditions of reduced blood flow (Ryan 1976). This offers a good explanation as to why the proteinuria observed during the heterologens

phase is so short lived despite the continuing presence of antibody. This mechanism does not however account entirely for the massive proteinuria observed - up to 1000 times the baseline level.

(b) It seems clear that the major factor causing increased permeability of the GBM to protein is the reduction of net negative charge on the capillary wall, increasing the permeability to anionic proteins such as serum albumin (Brenner 1978). It appears that antibody reacts with anionic antigenic sites either in the GBM, the slit pore membrane or the sialoprotien covering the epithelial foot processes, causing a reduction of the net negative charge. Rapid fusion of foot processes follows as a secondary morphologic event which is rapidly reversible (Couser 1976).

Many other experimental models demonstrating a similar phenomenon, have recently been described (Bennett 1976, Bohrer 1978, Vernier 1959, Seiler 1977).

It is therefore apparent that binding of antibody to the GBM may have widely variable effects ranging from no injury (when the antibody is directed towards the collagen component of the GBM alone) to polymorph dependant injury. The latter may or may not require the presence of complement. Finally, as discussed above, marked alterations in GBM permeability may result solely from the effect of

antibody binding.

Fc Related Effector Function

Most of the biologic effects following antibody binding are mediated through the Fc portion of the molecule. It appears that in the native state, Fab portions continually rotate around the hinge regions through an arc of about 30° (Gallager 1974). Binding to antigen restricts this movement and results in compacting of the molecule and exposure of areas on the Fc portion for binding to cellular Fc receptors, or to Fc binding proteins such as C1q (Winkelhake 1978).

Classes of antibody which bind C1q include IgM and IgG (Subclasses 1 & 3) as well as proteolytic fragments of IgA (Iida 1976). Intact IgA in contrast activates complement via the alternative pathway. This mechanism has been shown to be of importance in renal diseases such as IgA nephropathy and Henoch-Schonlein purpura (Meadow 1972). $F(Ab)_2$ fragments can also activate complement via the alternative pathway (Steele 1977).

The relationship of immunoglobulins to other inflammatory mediators is discussed below and also in Part V of this thesis.

1.3.3 The complement system

Studies of nephrotoxic nephritis (Review Unanue 1967) have demonstrated the importance of complement in the mediation of allergic renal injury. It binds to the GBM in association with nephrotoxic antibody (Hammer 1963) and causes amplification of injury by recruitment of other inflammatory mediators. Removal or inactivation of complement, by using cobra venom factor (CVF) or by using animals congenitally deficient in certain complement components, during the various phases of NTN, has further helped to define its role (Cochrane 1970).

During the heterologous phase of nephrotoxic nephritis in rabbits the type of injury is very dependant on the animal of origin and nature of the injected heterologous antibody. By using both complement binding and noncomplement binding antibodies with or without polymorphonuclear leucocyte (PMN) depletion, the role of these factors has been further defined. The most severe injury results from complement activation which allows for PMN attraction and, with antibody, the stimulation of lysosomal enzyme release. The latter damages basement membranes, and results in proteinuria. However, depending on the nature of the anti-GBM antibody, a considerable proportion of injury may result independently from PMN's and complement (Cochrane 1965, Cochrane 1979).

Complement binding in the absence of neutrophils does not

cause injury, demonstrating that the lytic action of the attack phase of complement does not appear to be of importance in glomerular injury (Cochrane 1979). Furthermore, Thomson (1975) has shown that injury during the autologous phase of NTN was as severe in non complement depleted animals, as in rabbits decomplemented with cobra venom factor (CVF). On the other hand , protection is provided by removal of circulating PMN's, illustrating their central role in this type of injury.

Certain immune complexes however, seem to require the presence of complement to cause proteinuria in leucocyte depleted animals (Salant 1978).

The failure to detect complement in the kidneys of 25% of cases of anti-GBM antibody disease (Wilson 1973) suggests that a similar mechanism of complement independent PMN dependent injury may operate in humans.

I.3.4 Polymorphonuclear leucocyte (PMN) dependent injury

Following the injection of nephrotoxic antibody, PMNs can be found in increased numbers in glomeruli and, on electron microscopy be seen to be displacing endothelial cells from the basement membrane (Cochrane 1965). These PMNs release lysosomal proteases which hydrolyse GBM proteins, causing increased vascular permeability and proteinuria.

Similarly PMNs play a central role during the autologous phase of NTN by causing damage as noted above. In addition the leak of fibrinogen into Bowman's space stimulates a proliferative reaction from epithelial cells and macrophages resulting in "crescents". Depletion of PMNs results in diminution of all aspects of injury during this phase (Naish 1975, Thompson 1975).

In experimental immune complex serum sickness, however, glomerular injury may occur in the absence of PMNs whilst the arteritic component of the injury is PMN dependent (Kniker 1965).

I.3.5 The coagulation system

Activated components of this system are involved in three processes: (a) the generation of kinins, (b) the formation of fibrinogen and (c) fibrinolysis, all of which are involved in some aspects of inflammation.

The important role of fibrin in the induction of crescents in the autologous phase of NTN was clearly demonstrated by Thomson (1975) using the defibrinating agent "Arvin". By contrast, depletion of fibrin during the heterologous phase appeared to have no effect on proteinuria (Naish 1975). Similarly, in acute and chronic serum sickness models (PMN independent injury) fibrin depletion also did not appear to play an important role (Naish 1975).

The exact role of these coagulation factors in human disease has not yet been established.

I.3.5 Mononuclear cells/macrophages

Monocytes have been identified in the glomeruli of animals and humans (Shigematsu 1973) with glomerulonephritis. Schreiner (1978), in an accelerated model of NTN in rats, noted the progressive replacement of PMN's in glomeruli by mononuclear cells 48 hours after induction of NTN. These cells were shown to have arisen from outside the glomeruli and were actively involved in the proliferative response. Proteinuria could be totally prevented by elimination of monocytes. In acute serum sickness anti-macrophage serum could produce similar protection (Holdsworth 1981). Atkins (1976) identified monocytes as a major component of extracapillary crescentic proliferation in human disease, a finding confirmed in experimental models of crescentic nephritis (Cattell 1978, Thomson 1979).

The stimuli that cause monocytes to enter the glomeruli have not yet been defined. It seems that local inflammation may be involved, although PMNs and complement do not appear to play a role (Cotran 1981). The infiltrate may partly be a response to T-cell stimulation similar to that seen in delayed hypersensitivty reactions (Unanue 1983).

In addition to their phagocytic functions monocytes also have powerful effector functions (Nathan 1980). They

release a vast range of secretory products, are essential for antigen processing and, in addition to bearing surface Fc and C3b receptors, also bear HLA-A,B,C and the immune associated Ia (DR) antigen.

Once in the glomeruli they cause derangement of function, possibly by the release of proteolytic enzymes. The proliferative response seen with the resident cellular population, may be due to release of growth promoting factors (Unanue 1983). In vitro they cause both endothelial and mesangial cells to proliferate, whilst Polverini (1977) showed that macrophages in culture released a factor which induces neovascularization. This is discussed further in Part I.4.3.

I.3.7 Platelets

For a variety of reasons the role of platelets in glomerulonephritis has been difficult to define. Depletion is difficult to achieve as animals tend to bleed to death before adequate depletion is achieved and great differences in platelet physiology and function exist between different species of animals. Furthermore, although human platelets have Fc receptors, unlike experimental animals, a C3b receptor is absent.

There is compelling evidence, however, implicating platelets in the causation of human disease. This includes

shortened platelet survival in glomerulonephritis (George 1974) as well as the finding of circulating platelet aggregates (Woo 1980), platelet membrane antigens and granule contents at sites of injury in glomeruli (Miller 1980, Duffus 1982).

Platelets are certainly very effective as inflammatory mediators: they react with immune aggregates via their Fc receptor (in humans) with resultant aggregation and release of contents (Henson 1973, Clark 1980, Shigematsu 1979).

Substances released from platelets include: proteolytic enzymes, vasoactive amines, many different prostaglandins, platelet factor 4 (chemotactic for polymorphonuclear leucocytes and monocytes) (Duvel 1981), platelet activating factor (acting on white cells) (Benveniste 1972), and B-thromboglobulin. The latter inhibits PGI_2 (prostacycline) production by the endothelium (Hope 1979).

Platelet derived growth factors are also capable of stimulating the growth of isolated glomerular cells (Nakashima 1980).

Experimentally, in the autologous phase of NTN, (anti-GBM antibody mediated) platelet depletion will reduce proteinuria although it does not appear to affect crescent formation, thought to be largely mediated by fibrin deposition (Sindrey 1979). They also play a role in promoting localization of circulating immune complexes in

rabbits (Benveniste 1972) and possibly also in humans (Cochrane 1971).

No firm conclusions can be drawn at this stage about their precise role in the pathogenesis of human glomerulonephritis or about the indications for the use of antiplatelet agents in these disorders.

1.4.0 THE FUNCTION OF THE MESANGIUM AND MONOCYTE PHAGOCYTIC SYSTEM (MPS) IN GLOMERULONEPHRITIS

1.4.1 Introduction

In studying glomerulonephritis considerable attention has been focused at the level of the glomerular basement membrane (GBM) where most of the important pathologic events occur. However, the function of the glomerular mesangium with its cells, matrix and population of phagocytic cells and the more distant function of the monocyte phagocytic system (MPS), may also have a profound effect on the genesis and perpetuation of many types of glomerulonephritis.

1.4.2 The glomerular mesangium

Significant abnormalities occur in the mesangium in association with disease states. For example in diabetic nephropathy mesangial matrix expansion with nodule formation occurs, and in immune complex glomerulonephritis

(GN), mesangial immune deposits, proliferation and increased matrix formation are found (review Michael 1980).

(a)Kinetics of macro-molecules in the mesangium.

It has become apparent that the glomerular mesangium is an important route for the transport of large aggregates and immune complexes (Michael 1980). Furthermore the presence of contractile elements in these cells (microfilaments and myosin - Scheinman 1974) as well as their proximity to the lacis cells of the juxtaglomerular zone, suggest a role in the regulation of the glomerular microcirculation. The mesangial matrix located between these cells although similar in some ways to the GBM is antigenically distinct. It consists of a collagen-like material containing some fibronectin (Scheinman 1978).

In experimental immune complex nephritis it has been shown that poorly soluble complexes of intermediate size tend to localize in the mesangium, (as do complexes containing high avidity antibodies) whilst smaller and more soluble complexes as well as those containing low avidity antibody, tend to localize in capillary loops (Germuth 1973).

Very soon after administration of anti-GBM antibody or the aminonucleoside puromycin, to rats, a striking increase of up to 10 fold, in the rate of uptake of administered aggregated IgG by the mesangium is seen (even before the proteinuria occurs). This effect is possibly due to the

loss of negative charge of epithelial cells allowing increased entry of complexes (Couser 1976). The rate of removal of these complexes from the mesangium however does not appear to be affected (Hoyer 1976).

It appears that at least two mechanisms for disposal of macromolecules from the mesangium exist (Velosa 1976, 1977). One seems to be the migration of complexes down the mesangial stalk to the juxtaglomerular zone and out into the interstitium. It is also possible for some regurgitation of complexes to occur back into the circulation, especially with ureteric obstruction (Raij 1979).

(b) Phagocytosis in the mesangium

Although ferritin is avidly taken up by mesangial cells, they probably do not participate in phagocytosis of immune complexes (Michael 1980). By contrast inflammatory cells such as polymorphonuclear leucocytes and macrophages may infiltrate the glomerulus and phagocytose actively. This issue is discussed further in the following section.

1.4.3 The monocyte phagocytic system (MPS)

Although it has been suggested that the glomerular macrophages originated in the glomeruli, it is now clear that they, like all other mononuclear phagocytes, are derived from bone marrow precursors (Crofton 1978).

The MPS plays a major role in clearing the circulation of immune complexes and in so doing decreases the chances of their localization in the kidney. Large immune complexes, containing more than two antibody molecules are removed rapidly with the disappearance rate described by a single exponential function (Mannik 1971). Complexes containing one or two antibody molecules are removed more slowly. Their disappearance from the circulation occurs both by equilibration between intra- and extra-vascular compartments and more slowly by catabolism (Haakenstad 1976). With large doses of immune complexes the Kupfer cells of the liver, which are responsible for removal of 90% of these complexes, become saturated. This results in prolongation of the half life of these complexes, and enhancement of their deposition in tissues, including the renal glomeruli, occurs (Haakenstad 1974).

Marrow-derived macrophages contribute to the removal of immune complexes from the mesangial matrix and the influx of these cells contributes to the hypercellularity observed. Schreiner (1978) demonstrated that these cells contributed to proteinuria and structural damage during the autologous phase of nephrotoxic nephritis in the rat. Furthermore they appear to constitute at least 50% of the cellular population of the crescents noted in various forms of experimental nephritis, including NTN in rabbits.

It has recently become apparent that the activity of Fc and C3b receptors on cells of the MPS may vary in different

disease states and that dysfunction may even perpetuate disease.

In a study by Jaffe (1978) patients with primary biliary cirrhosis, all had an abnormality of C3b receptor function but normal Fc receptor function. There was however, no correlation with the level of circulating immune complexes. In contrast patients with SLE had a marked defect of Fc receptor function which correlated very well with the level of circulating immune complexes (Frank 1979). The reason for this phenomon is not clear. Possibly the immune complexes depress MPS function, as is suggested by plasma exchange experiments in which return of MPS function follows rapidly after removal of circulating complexes by plasma exchange (Lockwood 1979). Alternatively the defect in MPS function may be primary, thus allowing for the persistence of immune complexes in the circulation.

Evaluation of patients with renal disease by Frank (1979) showed striking correlation between the presence of immune complexes, Fc receptor defects and the development of renal disease in patients with SLE. This is similar to the situation found in patients with Sjogren's syndrome and mixed cryoglobulinaemia who also manifest renal disease in association with clearance defects. In the latter two diseases however, striking levels of immune complexes may occur in the presence of normal Fc receptor function.

It would appear that the great diversity of expression of various immune complex mediated diseases depends not only on the level and different types of immune complexes which may exist in the circulation but also on the way in which they are handled locally in the kidney and by the MPS systemically.

I.5.0 THE RELATIONSHIP OF INFECTIONS AND ANTIBODY LEVELS TO SEVERITY OF DISEASE

I.5.1 Introduction

The clinical observation that patients with recurrent haematuria may relapse at times of acute infection was first made by Scheidemandel (1913) and Baehr (1926).

Subsequently it has become clear that many stimuli such as infection, trauma, vaccination and severe exercise may cause apparently enhanced injury in a wide range of conditions including such glomerulonephritides as anti-GBM antibody disease (Rees 1977, Johnson 1978), SLE (Dubois 1974), mesangial IgA disease (Clarkson 1977), Henoch Schonlein purpura (Day 1973, Cameron 1974, Meadow 1972), Wegener's granulomatosus (Pinching 1980), mesangio-capillary nephritis (Levy 1979), crescentic nephritis (Beaufils 1978) and minimal change nephropathy (Cameron 1974). It has also been noted in renal allograft rejection in humans (David 1972, Briggs 1972, Lopes 1973, Dosseter

1978, Byrd 1978,) as well as in other non-renal allergic diseases, myasthenia gravis and multiple sclerosis (Sibley 1965).

The phenomenon of infection-enhanced injury has received most attention in those forms of glomerulonephritis in humans and experimental animals characterized by circulating anti-GBM antibodies.

I.5.2 Human anti-GBM antibody disease

In human anti-GBM antibody disease a flu-like illness antedates the onset of renal or pulmonary disease in about half of the cases studied. It is not clear whether these patients already had some circulating antibody with the infection merely "triggering" a self perpetuating inflammatory reaction or whether by damaging lung basement membrane, the autologous anti-GBM antibody production is stimulated. The latter mechanism has been suggested in the 5-15% of patients who have a history of inhalation of volatile hydrocarbons prior to the development of this disease (Beirne 1977).

Similarly relapses of glomerulonephritis as well as pulmonary disease (haemoptysis) may occur without demonstrable rises in levels of circulating antibody (Rees 1977, Johnson 1978). Clearly factors other than merely antibody level are important.

As far as pulmonary disease is concerned, a particularly poor correlation has been noted between severity of disease and antibody level. An interesting additional factor is the association with cigarette smoking; 32 out of 35 patients with pulmonary haemorrhage were smokers compared to 3 out of 11 who were not (Cameron 1982).

I.5.3 Experimental anti-GBM antibody disease

Experimentally it has also been shown that in rabbits with an anti-GBM antibody type of glomerulonephritis, greater injury occurs in those that are infected when compared to those that are not (Rees 1981). Many experimental models have been established in an attempt to elucidate the mechanisms responsible for this phenomenon.

I.5.4 Possible mechanisms of enhanced injury

Virtually all the stimuli causing enhancement of injury also induce an acute phase response, the latter results in the supply of increased concentrations of humoral inflammatory mediators such as CRP, C3 and fibrinogen and in stimulation of cellular mediators such as polymorphs and macrophages. CRP is considered in detail in Part I.7.0 but it is worth noting here that it is a normal constituent of human and animal serum and that its concentration may rise 1000 - 2000 -fold after many stimuli, including infections (Pepys 1981). It affects the inflammatory response in many ways. It stimulates phagocytosis by PMN's (Kindmark 1971), it affects platelet function and the

release of vasoactive substances (Fiedel 1982), it activates complement by the classical pathway (Kaplan 1974, Osmand 1975) and has been shown to be present in vasculitic skin lesions (Parish 1976) and in some cases of SLE nephritis.

Infection generates increased quantities of circulating immune complexes, and may cause relapse when these complexes, which might be handled normally by healthy kidneys, localize and stimulate further inflammatory activity in the kidneys. This mechanism was suggested by Mauer et al (1972) who showed increased localization of immune complexes in kidneys previously "damaged" by small doses of NTG. A similar mechanism was demonstrated by Trevillian (1979) in a complex model where certain features of the acute serum sickness model (immune complex mediated damage) was superimposed on nephrotoxic nephritis (anti-GBM antibody mediated). This combination resulted in much more severe injury than would have been produced by either mechanism alone. In Wegener's granulomatosus, circulating immune complexes were only detected during infections associated with relapse, whilst infections not causing relapse, seldom had associated circulating immune complexes (Pinching 1980). It is however impossible to exclude the possibility that their presence might merely have represented reactivation of disease.

Infections might also affect the MPS, particularly its

ability to remove immune complexes from the circulation. Lockwood (1979) demonstrated impaired splenic function in patients with nephritis or vasculitis, which improved with plasma exchange, presumably by removing the circulating load of immune complexes, thereby allowing "recovery" of the MPS.

Not only do PMN numbers increase with infections (a very well known phenomenon) but qualitative changes in their function also occur. Exposure to endotoxin stimulates phagocytosis and enhances killing of staphylococci by PMN's (Cohn & Morse 1960), whilst a polypeptide isolated from E. coli has similar effects (Becker 1974).

Monocytes and macrophages undergo the greatest change during infections. Watson, Dixon and Feldman (1965) were able to enhance injury during the heterologous phase of NTN by giving Freund's complete adjuvant (FCA) two weeks before the nephrotoxic globulin (NTG), a manoeuvre designed to induce macrophage activation. Injecting incomplete Freund's or not allowing enough time for activation of macrophages and monocytes abrogated the enhancement of injury. A similar mechanism might explain the enhancement of injury which occurs in rats injected with subproteinuric doses of rabbit anti-rat NTG, when they recieve, by passive transfer, sensitized T cells from rats previously sensitized to rabbit gamma globulin (Bhan 1978).

Activated macrophages secrete increased amounts of neutral

proteases such as plasminogen activator, elastase and collagenase (Unkeless 1974, Werb 1975), whilst increased numbers of Fc receptors have also been found (Atkinson 1974). All these changes enhance the potency of the macrophages in inflammatory reactions and hence their potential for causing injury.

The exact role of all these mechanisms discussed above in causing non-specific enhancement of injury still needs to be defined.

I.6.0 THE ACUTE PHASE RESPONSE

I.6.1 Introduction

The possibility that enhancement of injury by stimulation of inflammatory mediator systems might occur has led to a consideration of the role of the acute phase response.

Tissue injury is central to the consideration of allergic renal disease. The immediate local response to injury is acute inflammation which has been studied in great detail. Much less clear, however, is the role of the systemic response to tissue injury, the so-called "acute phase response".

Virtually any stimuli causing injury will provoke this response such as an acute myocardial infarction, major surgery, infections and various forms of allergic tissue injury. It has also been described during pregnancy, soon after birth and associated with neoplastic states (Review Pepys 1981).

A vast range of alterations occur, only some of which have been studied in detail. One of the best known responses that follows injury, mediated by the action of endogenous pyrogen, is pyrexia (Bernheim 1979, Dinarello 1978). Endogenous pyrogen is similar, or identical to interleukin I, is elaborated by monocytes and macrophages of the MPS and appears to mediate many other acute phase responses (Bernstein 1978).

I.6.2 Systemic responses

(a) Reticuloendothelial function is depressed, possibly due to transient depression of fibronection (Saba 1980).

(b) Metabolic changes include changes in protein synthesis (Beisel 1977) and lipid metabolism (Blackburn 1977).

(c) Endocrine changes include increased rates of synthesis of glucagon, insulin, ACTH, cortisol, catecholamines, growth hormone, thyroid stimulating hormone, thyroxin, aldosterone and vasopressin (Beisel 1977).

(d) Trace elements. Elevations in serum concentration of copper, and depression of iron and zinc levels (Sobocinski 1979, Konijn 1977).

(e) Immunosuppresion is seen in various malignancies, and following major trauma and burns. Many aspects of the immune response such as diminished neutrophil and macrophage bactericidal activity (Alexander 1978, Wang 1980), lymphocyte hyporeactivity due to unknown plasma factors (Gatti 1971) and defective cell mediated immunity (Wang 1980) have been noted.

(f) Other factors found in acute phase plasma include inhibitors of leucocyte chemotaxis and factors augmenting adherence of granulocytes to surfaces (Lentnek 1976).

I.6.3 Acute phase proteins

The plasma levels of many proteins vary during an acute phase response. A useful classification is given by Kushner (1982).

1. Concentration increases by about 50%
   - Ceruloplasmin
   - C3

2. Concentration increases by about two- to fourfold
   - α1-acid glycoprotein
   - α1-antitrypsin
   - α1-antichymotrypsin
   - Fibrinogen
   - Haptoglobin

3. Concentration usually increases several hundredfold
   - C-reactive protein (CRP)
   - Serum amyloid A-protein (SAA)

Other proteins also increase to a variable degree. These include, factor B, total haemolytic complement activity, C2, C4, C5, 6, C9, (Alexander 1978), plasma ferritin (Daniels 1974,) angiotensinogen, kininogen and kininogenase (Elin 1977).

I.7.0 C-REACTIVE PROTEIN

I.7.1 Introduction

C-reactive protein as an acute phase protein receives much attention in this thesis both because of its capacity to increase impressively with an acute phase stimulus providing a useful indicator of the presence of an acute phase response and because of its possible role as an inflammatory mediator capable of modifying the severity of local inflammatory injury.

The appearance of CRP during acute inflammation was first described by Tillet and Francis (1930) whilst investigating patients with pneumonia whose sera were shown to precipitate the C-polysaccharide (CPS) fraction extracted from pneumococci. After recovery the capacity of the patients' sera to precipitate CPS rapidly disappeared. The factor responsible for this precipitation was CRP.

The term "acute phase" was coined by Abernethy and Avery (1941) to denote serum taken from acutely ill patients containing this CRP. They also showed the absolute requirement for calcium ions for its binding to CPS. Today it is realized that the CRP response is part of the general non-specific "acute phase reaction".

I.7.2 Kinetics of CRP synthesis

CRP is synthesized in the liver (Hurliman 1966), the cells

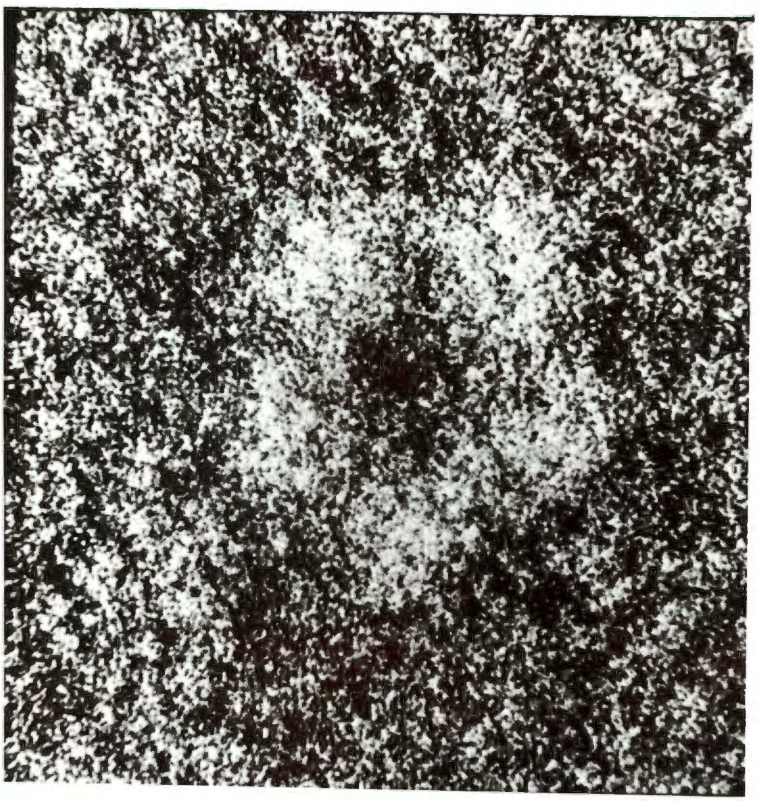
responsible being the hepatocytes, (Kushner 1978) and is normally present in the serum at levels ranging from 68 to 8100 ng per millilitre with a median value of 580 ng per millilitre (Claus 1976). The rate of synthesis increases within hours of injury (Kushner 1978) and may reach levels of 300 ug/ml within 24-48 hours.

The stimulus for increased production appears to be transmitted by humoral mediators such as leucocyte endogenous mediator (Merriman 1975), subsequently shown to be identical to Interleukin-I (Sztein 1981). Other possible mediators include prostaglandin PGE1 (Whicher 1980), activated complement components (Horowitz 1979) and possibly neuroendocrine factors (Turchik 1980).

I.7.3 Occurence of CRP

CRP has been identified in a large number of mammalian species (Pepys 1978, Oliveira 1980) and although it is not a noteworthy acute phase reactant in many animals, there is considerable similarity between human and rabbit CRP in relation to molecular weight, amino acid sequence (Oliveira 1979), kinetics following injury and binding properties (Oliveira 1980).

I.7.4 Structure of CRP



Electron micrograph of CRP molecule (After Pepys)

The CRP molecule consists of 5 non-glycosylated sub-units, each with a molecular weight of 21000 which are non-covalently associated in a unique disc-like configuration. Protein SAP (the p-component of amyloid) is the only other serum protein with a similar shape (Osmand 1977).

I.7.5 Functional properties

In addition to the calcium-dependent binding noted above, CRP also binds to choline phosphatides such as lecithin,

lysolecithin and sphingomyelin, to phosphoryl choline containing and non-phosphoryl containing polysaccharides present in bacteria, fungi and parasites. It binds to polyanions such as nucleic acids, heparin and dextran sulphate and to polycations such as histones, leucocyte cationic protein, myelin basic protein and protamine. (These latter reactions are not calcium dependent as a binding site other than the one for phosphoryl choline is utilized (Gotschlich 1982, Narkates 1982).

Of great interest recently is the finding that CRP binds to lipid bilayers. This raises the possibility that alteration of the normal phospholipid architecture of cell walls allows CRP binding which, followed by complement activation, may be a major mechanism for eliminating necrotic cells from sites of tissue injury (Narkates 1982).

Once CRP is bound either via its calcium dependent or polycation binding sites, it becomes a potent activator of the classical complement pathway (Volanakis 1982, Gewurz 1982). As a consequence it enhances phagocytosis and is involved in reactions of precipitation and agglutination (Gewurz 1982). It also affects platelet function by initiating reactions of aggregation and constituent release (Fiedel 1982) and binds to a small population of peripheral blood lymphocytes, the exact significance of which is as yet unknown (James 1982).

I.7.6 Clinical significance

An elevated CRP level is unequivocal evidence of a tissue damaging process and as such provides a useful screening test for many diseases such as Crohn's disease, polymyalgia rheumatica, rheumatoid arthritis (Amos 1977) and renal allograft rejection (Van Zyl Smit 1978).

Certain diseases may have striking symptoms; fever, an elevated ESR and other features of severe inflammation and yet have only a modest rise in CRP, for example, systemic lupus erythematosus, systemic sclerosis (Becker 1980), dermatomyositis and ulcerative colitis (Pepys 1981).

The basis for the differing CRP responses in different types of disease is not known. Possibly it may reflect genetic variation in the capacity to produce CRP. Such genetically determined differences in the capacity to produce CRP exist in certain strains of mice (Baltz 1980) and because of its capacity to act as an inflammatory mediator may affect individual susceptibility to, or manifestations of, the disease.

I.7.7 Role of CRP as a local inflammatory mediator

Of considerable interest to this thesis was the observation that CRP localized in inflammatory lesions induced by the intramuscular injection of typhoid vaccine (Kushner 1961), in experimental myocardial infarction in rabbits (Kushner 1963), and in certain types of cutaneous vasculitis (Parish

1976). Acute phase proteins have been shown to play a role in the pathogenesis of the Schwartzman phenomenon (Pepys 1982) and CRP injected intracutaneously in acutely ill patients produces a characteristic weal-and-flare reaction (Abernethy 1937).

Because of these observations, the possibility that CRP might also localize in inflamed and necrotic glomeruli and possibly modify the local inflammatory reaction was investigated in this thesis.

In an attempt to determine the severity of the acute phase response, when it was induced experimentally and when it formed part of the animal's response to the stimulus of nephrotoxic nephritis, the levels of CRP, fibrinogen and C3 were measured. The question whether the magnitude of the CRP response corresponded to the severity of injury was also considered.

PART II

PRODUCTION AND EVALUATION OF A MODEL OF NEPHROTOXIC NEPHRITIS IN RABBITS

PART II

PRODUCTION AND EVALUATION OF A MODEL OF NEPHROTOXIC NEPHRITIS IN RABBITS

II.0.0 INTRODUCTION

In part I, all the work done in establishing a reproducible model of nephrotoxic nephritis (NTN) in rabbits and which forms the basis for all the later studies, is described. This model is well known and generally accepted. My objectives therefore, were firstly to produce and test all the reagents needed to induce this disease in rabbits. Secondly, to determine optimal conditions for induction and study of nephrotoxic nephritis, such as dose response curves, optimal duration of study and reproducibility of results.

The following aspects of NTN were studied:

(a) The relationship of the dose of nephrotoxic globulin (NTG) given to the amount of proteinuria produced in both heterologous and autologous phases.

(b) The effect on renal function of heterologous and autologous phase injury.

(c) Changes in level of the "acute" phase proteins, C3, fibrinogen and CRP.

(d) Histopathology during both phases.

At the time of doing these experiments, to the best of my knowledge, no one had ever looked at the possible role of C-reactive protein in this disease or its response during various phases of NTN.

II.1.0 MATERIALS AND METHODS

II.1.1 Animals

Rabbits

Partially inbred male New Zealand white (NZW) rabbits (Froxfield Rabbit Company, Froxfield, Hants, U.K.) were used for all the early experiments. Subsequently "Swiss Hare Rabbits" obtained from The Cape Provincial Animal Centre were used both for experiments of nephrotoxic nephritis as well as for the raising of antisera.

Housing and diet

The animals were housed in metabolic cages when 24-hour urine samples were required. They were all fed on a standard pellet and water diet. In studies involving the use of radio-isotopes the following salts were added to the drinking water starting two days before the injection of isotope: potassium iodide 0.2 mg/l and sodium chloride 4.5 mg/l.

Collection of samples

After dilating the ear veins by placing a drop of xylene on the ear, blood samples were taken by partially cutting the marginal ear vein with a scalpel. Intravenous injec-

tions were given into this same vein after dilatation.

Urine was collected in metabolic cages. Where great accuracy in 24-hour urine collections was required, rabbits were catheterized both at the start and end of the 24-hour period.

Anaesthesia for operative procedures involved the use of either sodium pentobarbitone (Sagatal, MayBaker) 30 mg/kg body weight given intravenously, or the use of fentanyl (Hypnorm, Jansen) 0.25 - 1 ml/kg body weight intravenously or intramuscularly.

Rabbits were killed either by giving a lethal dose of sodium pentobarbitone or by exsanguination by cardiac puncture following anaesthesia.

Sheep

Two sheep were supplied by and housed at Northumberland Hall Veterinary College, Potters Bar, Herts, U.K. These were injected intramuscularly with a preparation of rabbit basement membrane for the production of anti-rabbit basement membrane anti-serum. Blood was taken from the sheep by jugular vein puncture.

Guinea-pigs

Outbred Duncan-Hartley guinea-pigs were used for the preparation of various types of anti-sera (to be described later.)

II.1.2 Storage of Samples

Blood was allowed to clot by standing at room temperature for 3 hours, then centrifuged at 2400 r.p.m. for 10 min and the serum separated. Alternatively, blood was collected into tubes containing dissolved EDTA calculated to produce a final concentration of 0.01 molar.

After separation, disodium azide was added to a concentration of 0.01% and samples were frozen at -20°C until time of analysis. Samples taken for measurement of immune complexes and those stored for long periods were kept at -70°C and thawed only once.

II.1.3 Preparation of Nephrotoxic Sheep Serum (NTS)

Rabbit glomerular basement membrane was obtained from Dr. N.M. Thomson. The method which he had used for its purification is included in the Appendix.

Using the method of Steblay (1962) approximately 25 mg of this particulate rabbit basement membrane preparation was incorporated into 2 ml of Freund's complete adjuvant (Difco Laboratories, U.S.A.) using a homogenizer.

Two sheep were injected intramuscularly every two weeks with this preparation. Blood samples were taken at the same time for determination of sheep anti-rabbit basement membrane antibody titres by an indirect immunofluorescent

technique (Part II.1.19).

After a total of 9 injections was given, a titre of 1/80,000 was obtained in both sheep.

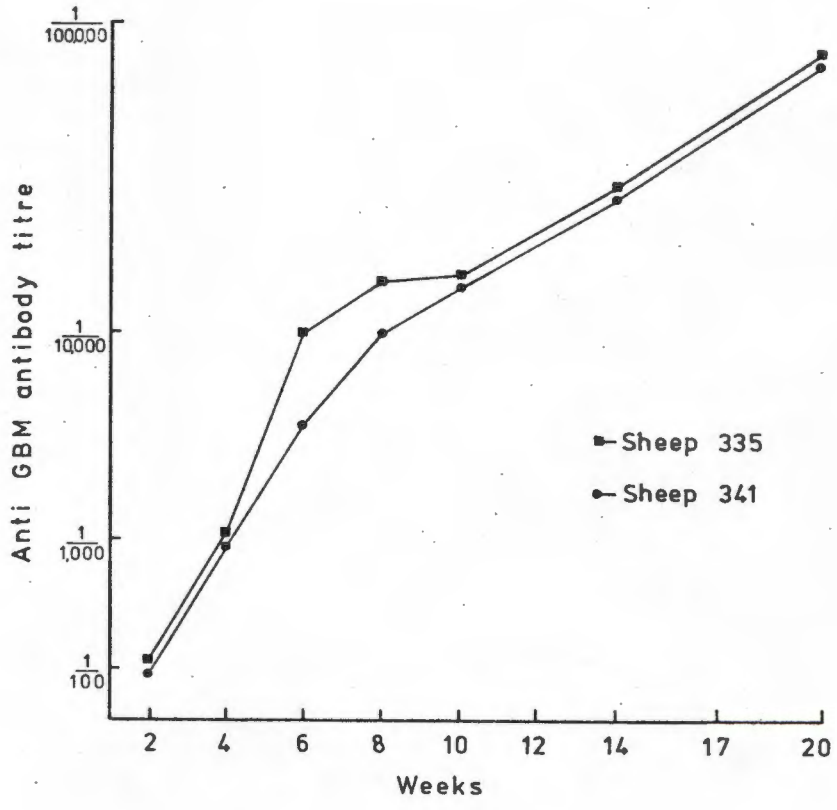


Fig I.1

The development of sheep anti-rabbit antibodies after repeated intramuscular injections of rabbit GBM emulsified with Freund's complete adjuvant.

The sheep were bled out and the blood was allowed to clot at room temperature for 4 hours, then overnight at 4°C. Sodium azide was added to a concentration of 0.01% as a preservative.

II.1.4 Purification of nephrotoxic serum

Nephrotoxic serum obtained as described above, contained agglutinating antibodies to rabbit red cells and formed a precipitation arc on double diffusion against normal rabbit serum (Ouchterlony 1953).

NTS was decomplemented by heating to 57°C for 30 min. Thereafter 2.5 ml of washed rabbit red cells were added to each 10 ml of serum and gently stirred for 30 min. The cells were then separated by centrifugation and the procedure repeated a second time as there was still some agglutinating antibody present.

Normal rabbit plasma was added in a ratio of 1:4 in an attempt to adsorb out antibodies to non-specific rabbit proteins. The precipitation arc on gel diffusion was diminished but not entirely removed.

II.1.5 Preparation of nephrotoxic globulin (NTG)

A globulin fraction of the NTS was prepared using the 50% ammonium sulphate precipitation method described by Heide and Schwick (1973).

A saturated solution of ammonium sulphate was slowly added to an equal volume of NTS stirring continuously. Stirring was continued for another 4 hours at 4°C to allow complete precipitation. This preparation was centrifuged at 3000

r.p.m. for 30 min and the precipitate was then washed in a 50% solution of ammonium sulphate. Thereafter it was redissolved in distilled water and the whole process of precipitation and washing repeated twice.

The precipitate was then dissolved in a small quantity of phosphate buffered saline (PBS) (pH 7.3), placed in cellophane dialysis tubing (Visken) and dialysed for 48 hours at 4°C with frequent changes of buffer (PBS) and constant stirring. The final preparation was centrifuged at 3000 r.p.m. for 30 min to remove insoluble debris.

PBS was added to restore the volume to that of the original NTS. All buffers used contained sodium azide 0.01%.

The protein content of the final preparation of NTG was 26 mg/ml as determined by the Folin method (Lowry et al 1951) - (Part II.1.18).

II.1.6 Preparation of normal sheep globulin

Serum taken from a normal sheep was processed in exactly the same way as the nephrotoxic globulin for subsequent use as a "plasma marker".

II.1.7 Radiolabelling of NTG and other proteins with ^{125}I and ^{131}I

A technique based on the method described by McConahy and Dixon (1966) was used.

The concentration of the proteins to be labelled was adjusted to lie between 20 and 30 mg/ml (dissolved in PBS pH 7.5). The exact concentration was determined by the Folin method (Part II.1.18).

The radio-isotopes ^{125}I and ^{131}I dissolved in sodium hydroxide pH 8.6 at a concentration of 1 mci/10 ul was obtained from Amersham Radiochemicals Ltd. Fresh solutions of chloramine-T (2.5 mg/ml distilled water) and sodium metabisulphite (2.5 mg/ml distilled water) were prepared immediately before use.

Method

One millicurie (or 500 uci) of radioactive isotope was added to 4 mg of protein (NTG) and mixed for 10 seconds. Chloramine-T was then added (15 ug/mg of protein) and was gently shaken for 60 secs. The reaction was stopped by the addition of sodium metabisulphite (30 ug/mg protein) with further shaking.

Further iodine was removed by passage of this preparation down a sephadex G25 column containing PBS pH 7.5 buffer.

If the percentage of free ^{125}I was greater than 1%, (see below for method of determination), the sample was dialysed in cellophane tubing overnight with frequent changes of buffer (PBS pH 7.5) until the percentage of free iodine was less than 1%. The efficiency of labelling was determined by comparing the free to protein bound ratio using a trichloracetic acid (TCA) method.

Determination of the percentage of radioactive iodine by TCA precipitation.

The volume of the sample to be precipitated was made up to 2 ml with a protein containing buffer (PBS + 20% BSA). An equal volume of 20% TCA (2 ml) was added to precipitate all the protein. The sample was centrifuged at 3000 r.p.m. for 20 min and the supernatant filtered through nylon wool. One ml of supernatant was counted and the percentage free iodine calculated as follows:

$$\% \text{ free iodine} = \frac{1 \text{ ml supernatant cpm} \times 2}{\text{Total cpm of sample}} \times 100\%$$

II.1.8 Preparation of antirabbit fibrinogen

The blood from one rabbit bled by cardiac puncture was taken directly into a trisodium citrate anticoagulant (31 g/l water mixed 1 vol : 9 vol blood). The blood was centrifuged immediately and the plasma volume measured.

Prothrombin was adsorbed twice with barium sulphate (5 gm % W/V). The mixture was stirred for 5 mins, then centrifuged and the precipitate discarded.

The plasma was now diluted with an equal volume of phosphate buffer (K_2HPO4 0.15 ml pH 7.2). Then twice the volume of the plasma of saturated ammonium sulphate (pH 7.0) was added dropwise, with constant stirring.

This mixture was allowed to stand at 4°C for 60 min to allow complete precipitation of the fibrinogen, then centrifuged at no more than 2000 r.p.m. The precipitate was washed twice in 25% saturated ammonium sulphate.

The precipitate was then redissolved in the original volume of KH_2PO4 buffer and re-precipitated with an equal volume of 50% saturated ammonium sulphate. Repeat standing and washing x 1.

The precipitate was redissolved and precipitated a third time and then redissolved in 0.3 ml potassium chloride + 0.05 ml sodium citrate pH 8.5 and dialysed overnight against 0.3 ml KCl at 4°C. The final solution was centrifuged, the precipitate discarded and protein concentration determined by the Folin method (Part II.1.18).

Twenty milligrams of fibrinogen solution was homogenized with an equal volume of Freund's complete adjuvant (FCA) (Difco) and injected intramuscularly into five guinea-

pigs. Two weeks later the procedure was repeated, using incomplete Freund's adjuvant. Two weeks after that, the animals were bled out, the serum collected and tested on an Ouchterlony plate. It gave a single line against rabbit plasma and no line against rabbit serum.

II.1.9 Preparation of anti-rabbit C-reactive protein

Serum was collected from rabbits in whom an "acute phase" response had been induced by the subcutaneous injection of a 1% croton oil in liquid paraffin mixture. This serum was subsequently shown to be rich in C-reactive protein.

Note: At the time of my early experiments (1977), I was unable to obtain pneumococcal C-polysaccharide required for the purification of rabbit CRP,as described in the method by Osmand (1975), nor had the review of purification techniques by Pepys (1977) been published. The CRP used for my initial experimets was purified as described below, but for my later experiments involving CRP, I used highly purified CRP prepared for me by affinity chromatography with pneumococcal C-polysaccharide by Dr.M.Pepys. (The method used is given in the Appendix.)

Adsorption to barium sulphate

C-reactive protein was extracted from this serum by the method of Ganrot and Kindmark (1969). The serum was

cleared by filtration, then to 1/10 of its volume a 20% (w/v) suspension of barium sulphate was added and mixed well with continuous stirring for 30 min. A fresh suspension of barium sulphate was prepared by adding concentrated sulphuric acid dropwise to a solution of barium hydroxide in water. This was continued until pH was just above 7. The mixture was then centrifuged at 1000 g for 10 min and the precipitate washed twice by suspending it in 0.9% sodium chloride.

The adsorbed proteins were eluted by suspending the barium sulphate in 1 litre of a 1.6 M ammonium sulphate solution (40% saturated at 20°C) stirring for 30 min, then centrifuging at 2000 r.p.m. for 15 minutes. Further ammonium sulphate was added to the supernatant until the concentration was 3.6 molar (90% saturation at 20°C). A fine precipitate formed. The solution was allowed to stand for one hour, then the proteins were collected by filtration in a Buchner funnel. The precipitated proteins were then dissolved in distilled water and dialysed against 0.04 M Tris-HCl buffer (pH 7.4) until no more ammonium sulphate remained.

Adsorption of CRP to agar gel

A 5% agar gel solution was prepared with Tris-HCl buffer (0.04 M Tris pH 7.4) containing 1 mM calcium chloride. An equal volume of this gel was added to the CRP-containing solution and was homogenized in a "Waring" blender. The

suspension was stirred at room temperature for one hour and then transferred to a Buchner funnel. The agar suspension was washed with the calcium chloride containing Tris buffer until the effluent was free of protein.

The CRP bound to the agar by calcium dependent bonds was now eluted using the same 0.04 M Tris buffer but now free of calcium and containing 0.02 M EDTA. The eluate was dialysed overnight against Tris (0.04 M). Afterwards the solution was concentrated by a factor of 10 using a Diaflo Ultrafilter (Amicon, Lexington, Massachusetts, U.S.A.).

CRP in the final preparation was detected by anti-human CRP (commercial) which cross-reacted with rabbit CRP. (At the time of these early experiments, no rabbit CRP or anti-CRP was available commercially).

Production of anti-rabbit CRP

Six guinea-pigs were immunized with the CRP-containing preparation by injecting 1 mg of protein homogenized with an equal volume of Freund's complete adjuvant (FCA) intramuscularly. Two weeks later a similar amount was injected - on this occasion suspended in Freund's incomplete adjuvant; after another two weeks the animals were bled out by cardiac puncture.

Double immuno-diffusion (Ouchterlony) was done on serum from rabbits which had been injected to produce acute

phase sera (Part III.1.0), using normal rabbit serum as control. The presence of anti-CRP was evident, but also many impurities.

Preparation of solid phase immuno-adsorbents

Normal rabbit serum was insolubilized by polymerization using the technique of Avrameas (1969). Normal rabbit serum was dialysed against 0.2 M acetate buffer (pH 5.1) overnight and the protein concentration determined (50 mg/ml). Twenty millilitres of this solution was stirred gently in a fume cupboard at room temperature, whilst 1.2 ml of ethyl chloroformate was added dropwise. The pH was maintained between 4.5 and 5 with 1 M NaOH. An abundant precipitate formed. The mixture was allowed to stand for 60 min. The precipitate was then washed once with 0.2 M glycine HCl buffer (pH 2.2) and thereafter with PBS (pH 7.4) until the pH of the sample was above 7.0.

Solid phase immuno-adsorption

Guinea-pig anti-rabbit CRP serum was added to the washed insolubilized normal rabbit serum and gently stirred for 1 hour at room temperature. The insoluble material was separated by centrifugation and this procedure repeated 3 times, each time checking that the level of antibodies to constituents other than CRP was diminishing. The final preparation contained only minute quantities of impurities and was successfully used for determination of CRP levels

in all the early rabbit experiments by radial immunodiffusion (Mancini 1965).

II.1.10 Preparation of anti-rabbit C3

A crude preparation of goat anti-rabbit C3 had previously been made in the renal laboratories of Hammersmith Hospital. The technique used involved the isolation of rabbit C3 using zymosan. The C3 coated zymosan was homogenized with Freund's adjuvant and injected directly into goats. They received a boosting dose two weeks later and after a further 2 weeks were bled out.

The preparation used gave 3 arcs on double diffusion in agar gel when tested against normal rabbit serum. These impurities were adsorbed out by adding 1/6 volume of rabbit serum from an animal decomplemented with cobra venom factor.

II.1.11 Purification of cobra venom factor (CVF)

Pure cobra venom CVF was prepared from crude venom extract by the method of Ballow and Cochrane (1969). Two grams of extract were dissolved in 100 ml of 0.01M phosphate buffer (pH 7.5 conductivity 1.8 mmho/cm^3) and dialysed against the same buffer for three days at $4^{o}C$. Neurotoxins were removed by fractionation on a DEAE-52 cellulose column, these being eluted in the breakthrough peak. The active CVF was contained in two very close peaks ,towards the end of a

saline gradient rising to 0.5M in three column volumes. Each fraction was tested for anticomplementary activity by determining its capacity to inhibit red cell lysis by the following test. A 0.1 ml aliquot of each fraction was added to 0.1 ml of 1/10 human serum (diluted in CFT) and incubated for 30 minutes at 37°C. Sensitised sheep erythrocytes were then added (0.2 ml of 1% solution) and incubated for 30 minutes. Fractions producing more than 50% lysis were pooled and concentrated to 30 ml by ultrafiltration. Phospholipase contaminants were removed on G-200 sephadex, equilibrated with 0.01M phosphate, 0.05 M NaCl (pH 7.2, conductivity 45 mmho/cm^3). CVF was contained in the first of two protein peaks which appeared early. Active fractions were pooled and concentrated by Amicon ultrafiltration.

The anticomplementary activity of the final preparation was determined by serial dilution, the degree of dilution maintaining greater than 50% lysis of red cells representing the number of cobra factor units per ml. I injected 300 units of this preparation into two rabbits and achieved total decomplementation within 48 hours.

II.1.12 Preparation of sheep IgG

Sodium sulphate precipitation

Sodium sulphate was added to 100 ml of normal sheep serum

until a concentration of 18% was reached. Stirring was continuous at 37°C for 3 hours. The precipitate was then centrifuged at 3,000 r.p.m. for 15 min, the supernatant discarded and washed 3 times in an 18% sodium sulphate solution. Finally the precipitate was redissolved in its original volume of PBS and then dialysed for 24 hours at 4°C against 0.01 M phosphate buffer (pH 7.6).

DEAE-cellulose chromatography

A 100 ml DEAE-cellulose column was prepared following the method of Fahey and Terry (1973). One gm dry weight of cellulose for each 50 mg of protein was used. Degassing was done at pH 4.0 using high molarity NaH_2PO4 (0.2 M). Then the pH was corrected with basic buffer Na_2HPO4 0.2M and finally the gel was equilibrated with 0.01 M phosphate buffer pH 7.6 (using a conductivity meter to ensure that column effluent was identical to eluting buffer).

The normal sheep globulin sample was now loaded onto the column and eluted with the 0.01 M phosphate buffer. Eluted fractions comprising the first protein peak were pooled and the protein concentration determined by the Folin method (Part II.1.18).

II.1.13 Preparation of human C1q

Purification was done using the method of Volanakis (1972) as modified by Zubler (1976).

Human blood was allowed to clot at room temperature for 2 hrs, then kept at 4°C for 6 hrs after which the serum was separated by centrifugation at 4°C and free lipid removed by centrifugation at 18,000 r.p.m. for 2 hrs.

To each 100 ml of human serum 25 ml of 0.1 M EDTA pH 7.5 was added and the pH adjusted to 7.5 at 0°C with 0.1 M HCl. The presence of EDTA ensures dissociation of C_{1q} from C_{1r} and C_{1s}.

This mixture was incubated at 37°C for 10 minutes and then cooled to 0°C and diluted to a relative salt concentration (RSC) of 0.04 (conductivity at 0°C of 2.75 m mho) (Zubler 1976).

The mixture was allowed to stand for 1 hr at 0°C with gentle stirring. The precipitate was recovered by centrifugation at 12,000 r.p.m. for 30 min and washed twice with 200 ml EDTA solution with RSC of 0.04 (conductivity of 2.75 mmho) pH 7.5. Afterwards it was dissolved in a small volume of 0.75 m NaCl, 0.01 M EDTA pH 5.0 and centrifuged to removed insoluble material at 12,000 r.p.m. for 30 min. The solution was then dialysed against three changes of 2,000 ml EDTA, RSC of 0.78 (conductivity of 5.55 at 4°C) pH 5.0. The precipitate formed was washed twice with 20 ml of the same EDTA solution and finally dissolved in 10.0 ml of 0.3 M NaCl, 0,01 M EDTA.

The entire procedure was repeated from the dialysis step.

This produced greater purity than would be achieved with only the two stages.

Insoluble aggregates were removed by centrifugation for 30 min at 20,000 r.p.m. The concentration of the C1q was determined by the Folin method (Part II.1.18) whilst the purity of the preparation was assessed by immunoelectrophoresis in 1% agarose to anti-whole human serum and by double immunodiffusion (Ouchterlony) using anti-human IgG and IgM. Small quantities of IgM contaminants were removed by the third precipitation step as described above.

II.1.14 Lactoperoxidase labelling of C1q with ^{125}I

Radio-iodination of C1q was done according to the method of Heusser (1973).

The concentration of C1q was adjusted to 1 mg/ml (in 0.3 M NaCl, 0.01 M EDTA pH 7.5). One millicurie (mci) of ^{125}I was added to 1 ml of C1q, then 25 ul lactoperoxidase (1 mg/ml in veronal buffered saline, VBS) was added, followed by 25 ul hydrogen peroxide diluted 1/10,000. Reagents were mixed and allowed to stand at $4^{o}C$ for 15 min. The reaction was stopped by the addition of 50 ul of a solution containing NaI 6 mg/ml and sodium azide 30 ug/ml.

This freshly labelled solution was then passed through a Sephadex G25 column equilibrated with NaCl 0.3 M, EDTA

0.01M plus 0.01 % sodium azide. The protein fractions were collected in the breakthrough peak and the percentage of remaining free iodine determined as described in Part II.1.7.

The ^{125}I C1q had a specific activity of about 1 uCi/ug protein.

II.1.15 C1q binding assay for circulating immune complexes

The ^{125}I labelled C1q was diluted in VBS containing 1% BSA and 0.05% Tween 20 with the volumes adjusted to give 100,000 cpm in a gamma counter, of each 50 ul of solution to be used. It was then centrifuged at 18,000 r.p.m. for 20 min to remove large aggregates. The test was done in LP3 polypropylene tubes, 50 ul of test serum was mixed with 100 ul EDTA 0.2 M pH 7.5 and incubated at 37°C for 30 min and then transferred to an ice bath. Fifty microlitres of ^{125}I C1q was then added and mixed well. Then 1 ml of 3% (W/V) polyethylene glycol (PEG) molecular weight 6000 was added. PEG was dissolved in 0.1 M Boric acid, 0.025 M disodium tetraborate, 0.075 M NaCl pH 8.3. Following the addition of the PEG, the mixture was again mixed and allowed to stand on ice for 60 minutes and then centrifuged at 2000 r.p.m. for 20 min. The supernatant was discarded and the radioactivity in the precipitate was measured and expressed as a percentage of the total radioactivity in the precipitate of a control tube, in which 1 ml of 20% trichloracetic acid (TCA) was added to 200 ul normal human serum (NHS)

mixed with 50 ul of ^{125}I C1q.

Heat aggregated rabbit IgG was used as a positive control and normal rabbit serum as a negative control. All estimations were done in duplicate.

II.1.16 Assessment of the capacity of human C1q to bind to rabbit IgG

Rabbit IgG at concentration of 5 mg/ml was aggregated by heating to 63°C for 30 min in a water bath. Doubling dilutions of aggregated rabbit IgG were made in normal rabbit serum.

The C1q binding assay as described above was done on all these samples. The results shown in figure II.2 show that human C1q bound effectively to altered rabbit IgG and could therefore be used to detect complement fixing circulating immune complexes in rabbit serum.

Fig.II.2 /.....

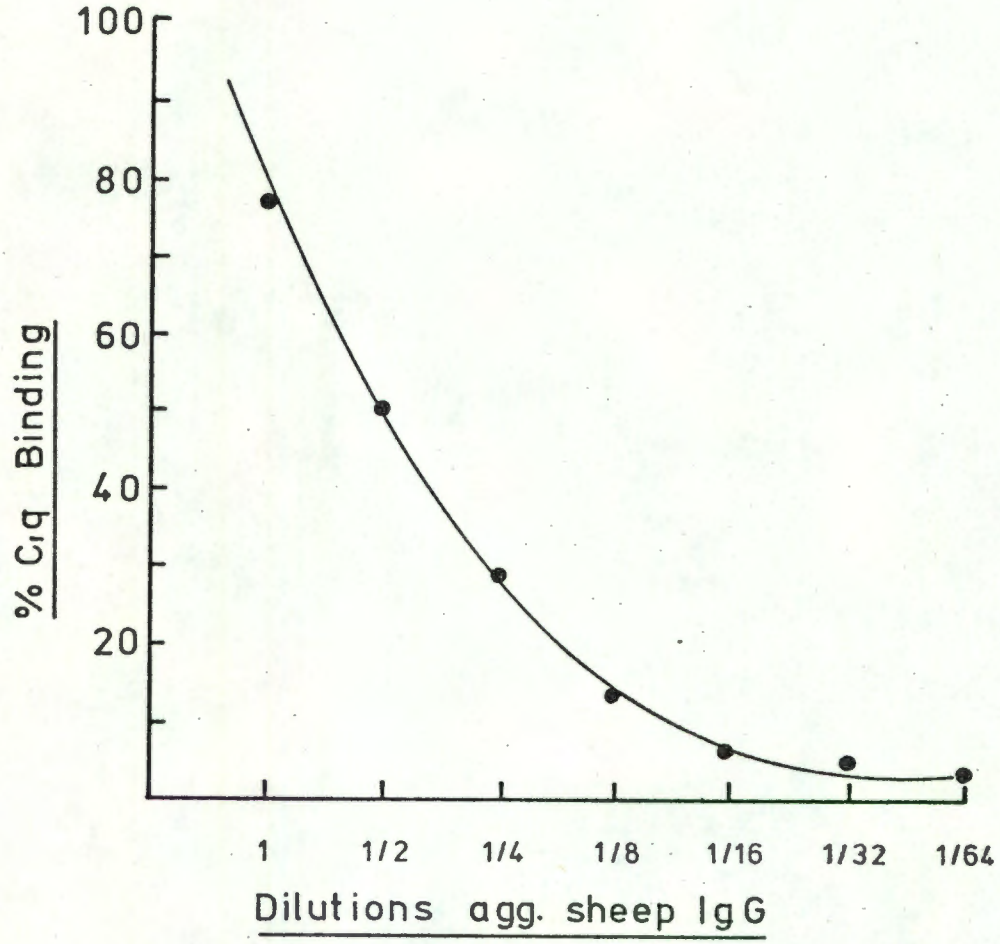


Fig.II.2

Human C1q binding to serial dilutions of heat aggregated sheep IgG.

II.1.17 Haemagglutination assay for determination of sheep anti-rabbitimmunoglobulin titres

Glutaraldehyde preparation of red blood cells (RBC's)

Human group O RBC's were taken into a citrate phosphate

dextrose buffer (Trisodium citrate 26.3 gm, citric acid 3.27 g, water 1 litre) using a dilution of 1:7 RBC's were washed three times in phosphate buffered saline pH 7.4.

Normal rabbit serum was decomplemented by heating to 56°C for 30 min. A 1% solution was made up with PBS and adsorbed against washed human group O RBC (ratio of RBC's : NRS = 1 : 2 incubated at 37°C for 30 min and then centrifuged).

Glutaraldehyde was diluted to 2.5% with normal saline.

Method (to make 5 ml of 1% treated RBC's)

Fifty ul of washed human group O RBC's were added to 1.25 ml of PBS containing 0.4 mg/ml of sheep IgG and mixed for 10 minutes. These concentrations were chosen after numerous trials had shown them to be optimal for this assay. Lower concentrations of sheep IgG lacked sensitivity, whilst higher concentrations caused auto-agglutination of the red cells. With each new preparation of sheep IgG the whole test was re-standardized. Then 0.25 ml glutaraldehyde (2.5% in saline) was added and mixed constantly for 1 hour at room temperature. These cells were washed twice in PBS, then once in 1% rabbit serum saline. Finally a 1% suspension of RBC's was made.

Haemagglutination assay

1. Doubling dilutions of the test sample in 50 ul of 1% RSS (rabbit serum saline) were placed in microtitre plates.
2. 25 ul of a 1% suspension of treated RBC's was added.
3. Controls consisted of the following:
   - doubling dilution of normal rabbit serum
   - a positive control using doubling dilutions of a known high titre rabbit anti-sheep serum
   - 3 standards consisting of dilutions of the high titre "positive control"
   - a further control using a 25 ul suspension of RBC's treated with glutaraldehyde but without the addition of sheep IgG.

After addition of the RBC's, the plates were gently agitated and allowed to stand at room temperature. Reading of the plates was done after 4 hours and after standing overnight. All assays were done in duplicate and the titre expressed as the mean of the two results.

II.1.18 ASSAY PROCEDURES

(a) Protein concentration (Biuret method)

Insoluble material was removed from urine samples by centrifugation at 2500 r.p.m. for 10 min. BSA protein standards of concentrations 40, 20, 10, 5, 1, 0.5 and 0.1 mg/ml were prepared in volumes of 1 ml. To each 1 ml of urine test samples and standards, 1 ml of a 20% trichloracetic acid (TCA) solution was added and mixed. Samples

were centrifuged at 3,000 r.p.m. for 20 min, the supernatant discarded and then the protein containing precipitate was dissolved in 0.5 ml of 1 M sodium hydroxide. After adding 2.5 ml of distilled water and 5.0 ml of Biuret reagent, the solution was mixed and allowed to stand for 30 minutes.

The optical density of the samples at 540 nm was read on a spectrophotometer using 3 ml of distilled water and 5 ml of Biuret reagent as a blank for zero optical density. The values of the standards were plotted and the concentration of the test samples read from the line.

(b) Protein concentration (Folin method)

Protein standards of 20, 50, 100, 150 and 200 micrograms of bovine serum albumin (BSA) were prepared each in 1.0 ml of distilled water. The solution to be tested was divided into aliquots of 50, 100, 150 microlitres and each made up to 1.0 ml with distilled water.

Folin A solution (1.0 ml 2% sodium potassium tartarate, 1.0 ml 1% copper sulphate, 100 ml 2% sodium carbonate in 0.1 M sodium hydroxide) was added to each tube (3.0 ml) and allowed to stand for 10 min at room temperature. Then 0.3 ml of Folin B solution (Folin and Ciocalteu's phenol reagent, diluted 1 in 2 in distilled water) was added and left for a further 30 min.

The optical density of the samples was read at a wavelength of 750 nm on a spectrophotometer. The values of the standards were plotted, a best fit line was drawn and the concentration of the test solution read from this line.

(c) Radial Immuno-diffusion

The concentrations of CRP, C3 and fibrinogen were measured using this technique described by Mancini et al (1965).

The specific anti-sera (to C3, CRP and fibrinogen) were added in optimal amounts to a 1.5% solution of Agarose (BDH Biochemicals, U.K.) in PBS BDTA 0.01 M (pH 7.4). The strength of the anti-sera varied greatly and optimal amounts ranging from 100 ul to 500 ul had to be determined and were added to 10 ml of agarose solution at 60°C. This was immediately poured onto a 10 cm x 10 cm glass plate and allowed to cool. Wells of 3 mm were punched into the agar and filled with 10 ul of test serum. Standards in the case of C3 determinations were obtained from pooled normal rabbit serum and expressed at 100%. CRP and fibrinogen of known concentration was available and was used to make up standards at dilutions of 100%, 75%, 50%, 25% and 10%.

After filling of the wells, the plates were left for 24 hours at room temperature in a humid environment for diffusion to occur. Thereafter the plates were washed in PBS for a further 24 hours, the gels were dried with blotting paper and the precipitation rings stained with Coomassie

blue.

The diameters of the precipitation rings were measured, the values squared and plotted against the concentrations. The values of the standards allowed a line to be drawn from which the values of the test sera could be read. In the case of C3, levels were expressed as 100% of the normal pooled serum whilst CRP and fibrinogen levels were expressed at mg/ml. All estimations were done in duplicate and the mean was taken as the final result.

(d) Double immunodiffusion

A 1.5% 1 Agarose in PBS gel was used. EDTA 0.01 M was added for all reactions involving CRP as it binds to agarose in the presence of calcium. The method used was that described by Ouchterlony (1953, 1958). A central well of 5 mm was cut and at 4 mm distance from the centre well, further wells of 3, 5, 6 and 8 mm. The antiserum was placed in the central well and the antigen containing solution in the peripheral wells. The plates were left at room temperature for 24 hours, then washed, dried and stained as described above; the precipitin lines were then assessed.

(e) Gamma counting

The radioactivity of samples containing both ^{125}I and ^{131}I isotopes was measured in a Packard gamma counter. Two channels were available for simultaneous measurement of

energy emission for each isotope. By choosing the appropriate "windows", the energy spectrum of the ^{125}I could be totally excluded from the ^{131}I channel whilst there was a crossover of about 20% of the ^{131}I counts into the ^{125}I channel. By determining the percentage crossover at the start of each counting run, the appropriate adjustments could be made to the ^{125}I counts.

The counting times varied from 0.5 to 20 min depending on the activity of the sample. Background subtraction was done in all cases.

(f) White cell counts

Total white cell counts were done on a Coulter counter whilst differential leucocyte counts were done manually on May-Grunwald-Griemsa stained smears.

(g) Urea and creatine determinations

Urea was initially measured by the microurease method (see below) but subsequently both urea and creatine were measured by auto-analyser techniques by technicians in the renal laboratories of both Hammersmith and Groote Schuur Hospitals.

Microurease method for urea determination

The following solutions were prepared:

1. Standard urea in distilled water 500 ugm/ml.

2. Urease solution. Disodium EDTA 3.0 gm was added to 0.2 gm of sodium nitroprusside and 0.2 gm of urease (Sigma type III). Thereafter it was blended in a mortar with a pestle, and kept in a dark bottle in order to prolong its shelf life. Immediately before use, 50 mg of this mixture was dissolved in 20 ml distilled water.

3. Alkaline hypochlorite solution. Twenty microlitres of 10-14% hypochlorite was added to 12.5 ml of 2% sodium hydroxide solution and volume made up to 1 litre with distilled water.

Method: One millilitre of urease was added to 10 ul of the samples and a blank. This was incubated for 15 mins at 40°C, then 2 ml of 2% phenol was added and 2 ml alkaline hypochlorite solution. Marbles were placed on the top of the tubes which were boiled for 5 min and then cooled for 10 minutes.

The optical density was read at 63 nm, a standard curve constructed and the volume of the test samples read from it.

II.1.19 Determination of the amount of anti-GBM antibody bound in the kidney

Nephrotoxic globulin was labelled by the chloramine T method as described (Part II.1.7). Normal sheep globulin prepared in the same way was labelled with ^{131}I and then added to the NTG in an activity ratio of 1 : 4. The total dose of isotope given to each rabbit intravenously was 1-3 uci. All rabbits had potassium iodide added to their drinking water as descibed above (Part II.1.1) to minimize uptake of radio-iodine by the renal tubular cells.

On removal of the kidneys, the cortex and inner medulla were separated and the latter discarded. One of two techniques was then employed. Either the kidneys were cut in small pieces, or the whole kidney was homogenized, and then washed three times in PBS and counted. Both methods gave similar, equally reproducible results. The left and right kidneys were analysed separately.

The ratio of ^{125}I and ^{131}I counts in the serum at the time of removal of the kidneys was determined by counting 2 ml of this serum. The ^{131}I labelled normal sheep globulin served as a marker for non-specific trapping of NTG in the kidney.

The amount of nephrotoxic antibody (NTab) bound in the kidney was calculated using the following formula:

$$\frac{\text{ug NTab}}{\text{per}}\ \underline{\text{kidney}} = \frac{{}^{125}\text{I kidney} - \left[\frac{{}^{125}\text{I/ml serum}}{{}^{131}\text{I/ml serum}}\right] \times {}^{131}\text{I kidney}}{{}^{125}\text{I counts per ug of NTG injected}}$$

The left and right kidneys were done separately and results expressed as ug NTab bound per kidney (mean of left and right) or ug NTab per gram of kidney tissue.

II.1.20 Microscopy

Light microscopy

Renal biopsy specimens were fixed in 10% formalin, in buffered saline, embedded in paraffin wax and sectioned at 4 u. Sections were stained with haematoxylin and eosin (H & E) and methenamine silver.

Immunofluorescent microscopy

Renal biopsy specimens were taken and immediately embedded in Tissue - Tek OCT compound (Ames Company, U.K.) on a small piece of cork. This was snap frozen without delay by immersion in liquid nitrogen and then stored at -70°C until examined.

A cryostat (Slee, London) was used to cut 4 u sections which were placed on glass slides. These were first dried in air and then fixed by immersion in dry acetone (prepared by addition of aluminium sodium silicate molecular sieve

type 4A - BDH Chemicals) for 15 mins. The sections were then washed three times for 10 mins in PBS (pH 7.4).

Fluorescein labelled antiserum in the appropriate dilution was dropped onto the sections to occlude them completely and left in a humid atmosphere at room temperature for one hour. All excess stain was then washed off by immersion in PBS (with gentle stirring) three times for 10 mins. The sections were then dried in air and mounted in 95% glycercol in PBS and kept in the dark until time of viewing.

A Leitz Orthoplan microscope using a high pressure mercury light source (HBO-200), K-500 FITC interference filter, BG-38 red suppression filter and a K510 or K530 barrier filter were used. Water immersion objectives of magnification 25x and 50x were used.

Electron microscopy

A Hitachi H 600 electron microscope was used. Samples were fixed in 5% phosphate buffered glutaraldehyde, then osmicated in "Palades" fixative and dehydrated with acetone, finally they were imbedded in "Spurs" resin and cut on a LKB Ultratome III. Sections stained with uranyl acetate and lead citrate were then viewed on the Hitachi microscope.

II.I.21 Statistical analysis of results

Linear Regression Analysis

This method was used to compare the dose of NTG given to rabbits, to the quantity of NTAb fixed in the kidneys and for the comparison of the quantity of NTAb fixed, to proteinuria. (Snedecor, 1956)

Wilcoxon Rank Sum Test for Two Samples

This method of analysis of unpaired measurements as described by Wilcoxon (1945) and modified by White (1952) for groups of unequal sizes, was used for the statistical analysis of most data, as indicated in the legends.

The statistical programs of the Department of Medical Informatics of Groote Schuur Hospital were used for the analysis of the remaining data.

II.2.0 EXPERIMENTAL PROTOCOL - HETEROLOGOUS PHASE.

Rabbits were housed in metabolic cages and received Iodine containing drinking water. For two days before the start of the experiment, baseline 24 hour urine excretion was measured, as was the C3, fibrinogen, C-reactive protein (CRP), creatinine and urea. Thereafter daily measurements were done as described in Part II.1.1.

The groups of rabbits each received an intravenous bolus infusion via an ear vein, of nephrotoxic globulin (NTG), with the doses ranging from 0.125 ml/kg body weight to 2.0 ml/kg body weight. The NTG was labelled with ^{125}I at an activity of 0.055 uci of ^{125}I per 1mg NTG. Normal sheep globulin, labelled with ^{131}I at an activity of 0.020 uci per 1mg was added, using 1/10 the volume of the NTG as a non specific plasma marker.

After 24 hours the rabbits were catherized to ensure adequacy of the urine collection and after 48 hours they were killed, again making sure that all the urine had been collected. Blood samples were taken at 24 and 48 hours.

The kidneys were removed and prepared for gamma counting of the bound isotopes, whilst a small piece was taken for light, immunofluorescent, and electron microscopy).

II.3.0 RESULTS - HETEROLOGOUS PHASE

II.3.1 Relationship between Dose of NTG given and Amount of Antibody fixed.

A group of 10 rabbits was given NTG according to the above protocol. As can be seen from Fig.II.3 and table II.1 a very close linear relationship was demonstrated between the amount of NTG given and the amount of nephrotoxic antibody fixed in the kidney. (Correlation coefficient = 0.99 ; slope = 3.73 ;) The mean percentage of NTG which bound was 0.35 % , range 0.33 % - 0.40 % .

Virtually 95 % of antibody binding takes place within the first 30 minutes. However as the antibody may cross react with structures such as choroid plexus, lung basement membrane and testis, the exact percentage of NTG which was made up of specific anti-GBM antibody, could not be determined by looking merely at GBM-bound antibody.

Fig. II.1 /.....

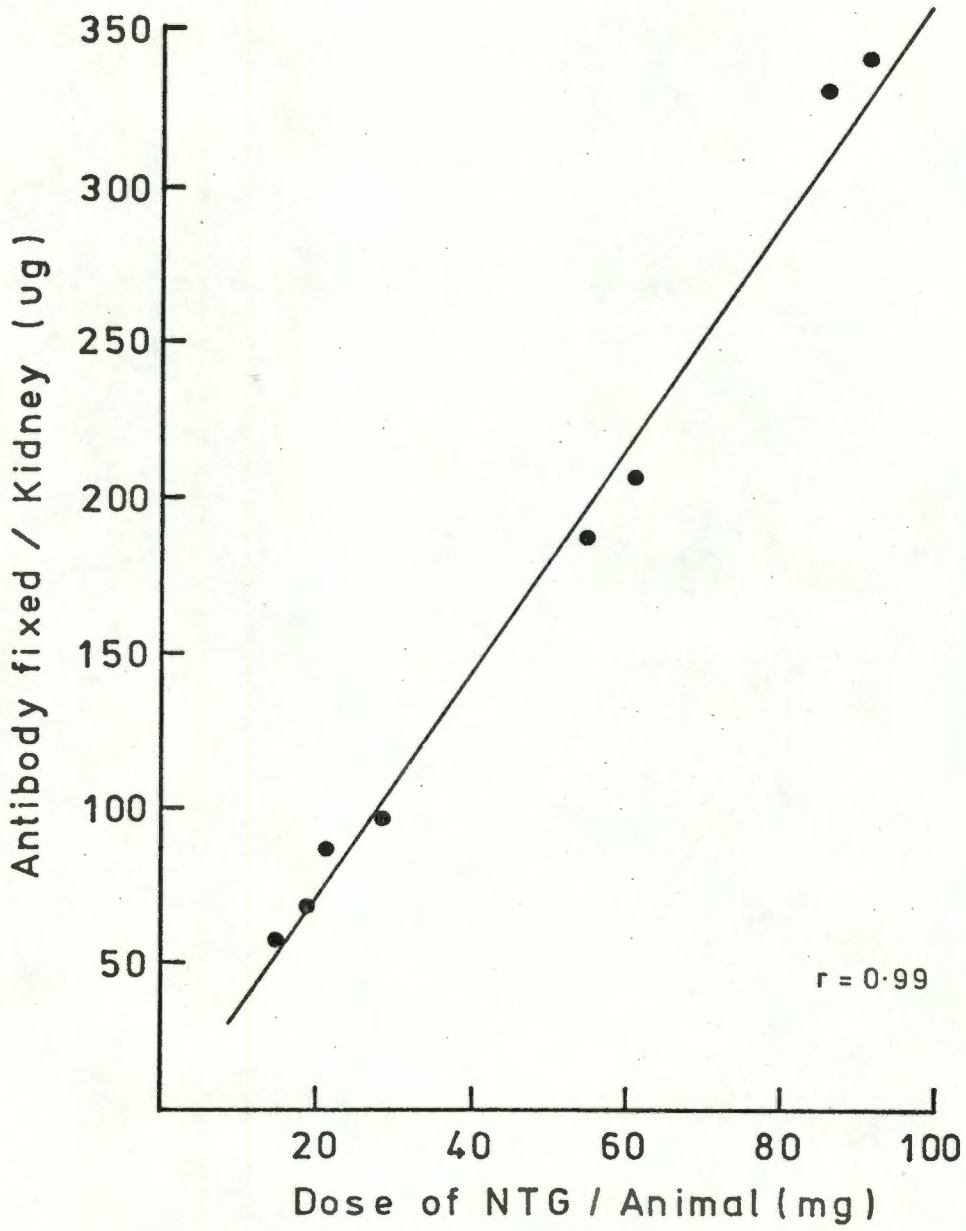


Fig. II.3

The relationship between the amount of NTG injected and the amount of nephrotoxic antibody fixed in the kidney. (correlation coefficient, 0.99, slope, 3.73) The mean percentage of NTG which bound was 0.35 % (range 0.33 - 0.40 %)

Table II.1 /...

DOSE		BINDING	
ml/kg	mg (total)	ug/kidney	%
2	85,80	330	0,38
2	91,80	340	0,37
1	61,50	205	0,33
1	55,50	185	0,33
0,5	21,25	85	0,40
0,5	28,50	95	0,33
0,25	19,50	65	0,33
0,25	14,85	55	0,37

Table II.1

The relationship between the dose of NTG given and the amount of antibody fixed. Doses calculated on ml/kg body weight basis. Mean percentage of NTG which bound was 0.35 % (range 0.33 % - 0.40 % ; SD = 0.03.)

II.3.2 The relationship between the amount of NTab fixed and Proteinuria produced.

Few rabbits developed proteinuria when less than 150 ug of antibody fixed per kidney. However when more than 200 ug of antibody bound , proteinuria always resulted.

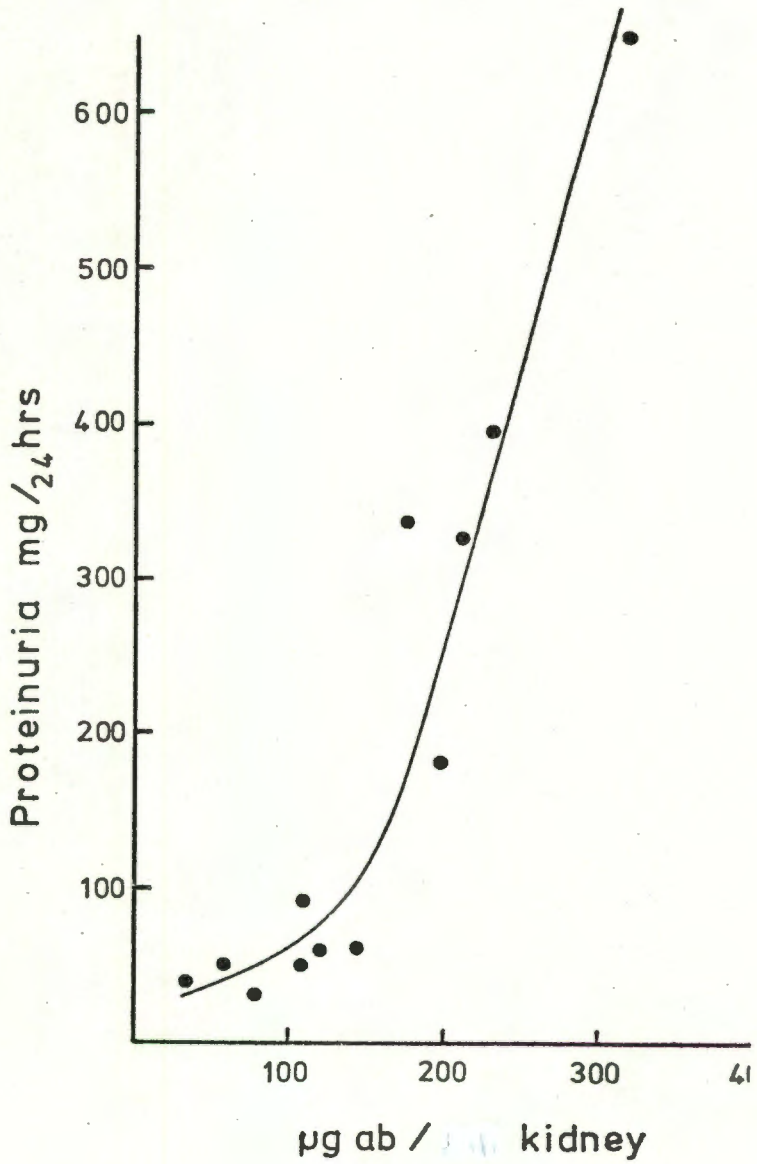


Fig. II.4

The relationship between the amount of nephrotoxic antibody bound and resulting proteinuria. (NTG prepared from sheep "341")

Rabbit	Proteinuria mg/24 hrs	µg Antibody per kidney
10	650	320
11	396	236
12	324	212
13	60	148
14	336	176
15	180	200
16	60	120
17	50	109
18	95	110
19	50	60
20	46	40

Table II.2

Relationship between amount of nephrotoxic antibody bound and proteinuria. The "threshold" exists between 120 ug and 200 ug of antibody bound per kidney.

Differences were apparent between different batches of NTG prepared from different sheep. Fig.II.5 shows a somewhat different dose response curve when a batch of NTG prepared from a different sheep was used.

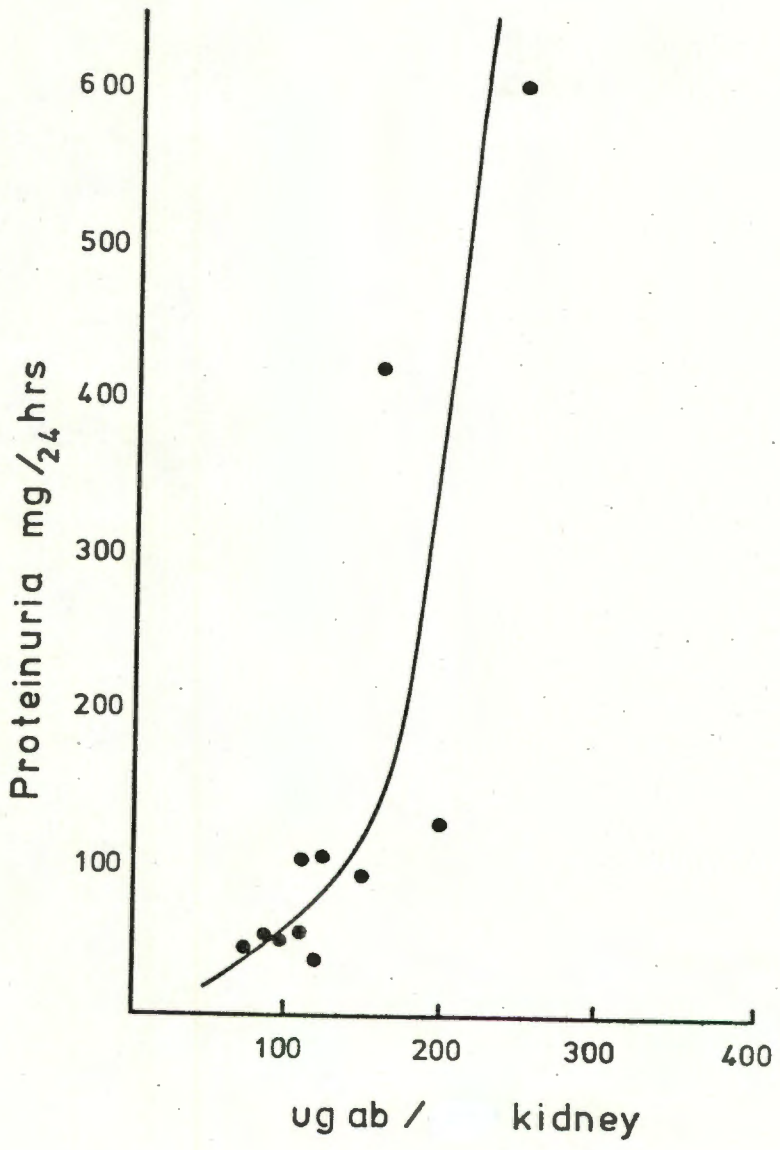


Fig. II.5

Relationship between the amount of NTab fixed and the resulting proteinuria. (NTG prepared from sheep "335")

During subsequent experiments involving 20 rabbits, when 13 mg /kg NTG was used resulting in the fixing of about 100 ug NTab per kidney, no rabbits developed significant proteinuria during the heterologous phase. This dose was specifically selected for experiments in which doses below the "threshold level" were required.

II.3.3 The effect of hypocomplementaemia on the binding of nephrotoxic antibody.

As considerable attention is being focussed in this thesis on the role of "acute phase proteins", the effect of low levels of complement (C3) on the binding of NTAb to the GBM was assesed.

Thomson (1975) showed that complement depletion did not affect the immediate binding of complement to the GBM. However, in view of the capacity of complement to solubilise immune complexes (via the alternative pathway), it was important to asses the effect of complement on the turnover of NTAb on the GBM, during the heterologous phase.

Rabbits were paired and given the following doses of NTG: 2.0 ml/kg; 1.0 ml/kg, and 0.5 ml/kg b.wt. Twenty four hours later, one rabbit in each pair was given 150 u of cobra venom factor (CVF) intravenously to induce decomplementation.

The effect of the CVF is shown in Fig.II.6

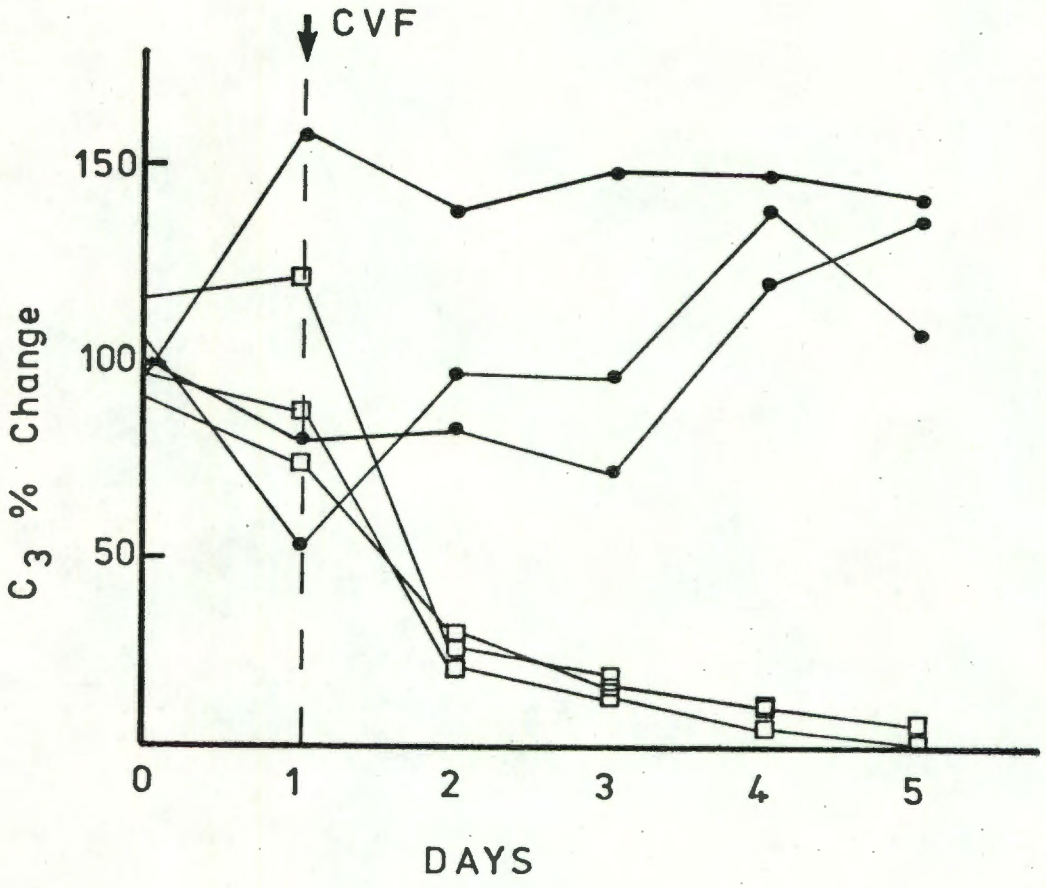


Fig.II.6

The effect of cobra venom factor CVF given during the heterologous phase on C3 levels. Control rabbits given only NTG (●), test rabbits received 150u CVF on day 1 (□)

On day 1 all rabbits had one kidney removed, four days later, the remaining kidneys were removed and the amount of NTAb bound determined as described in Part I.19. As seen from Fig.II.7, there was no difference in the amount

of NTAb fixed in the kidneys of the decomplemented animals as compared to the controls.

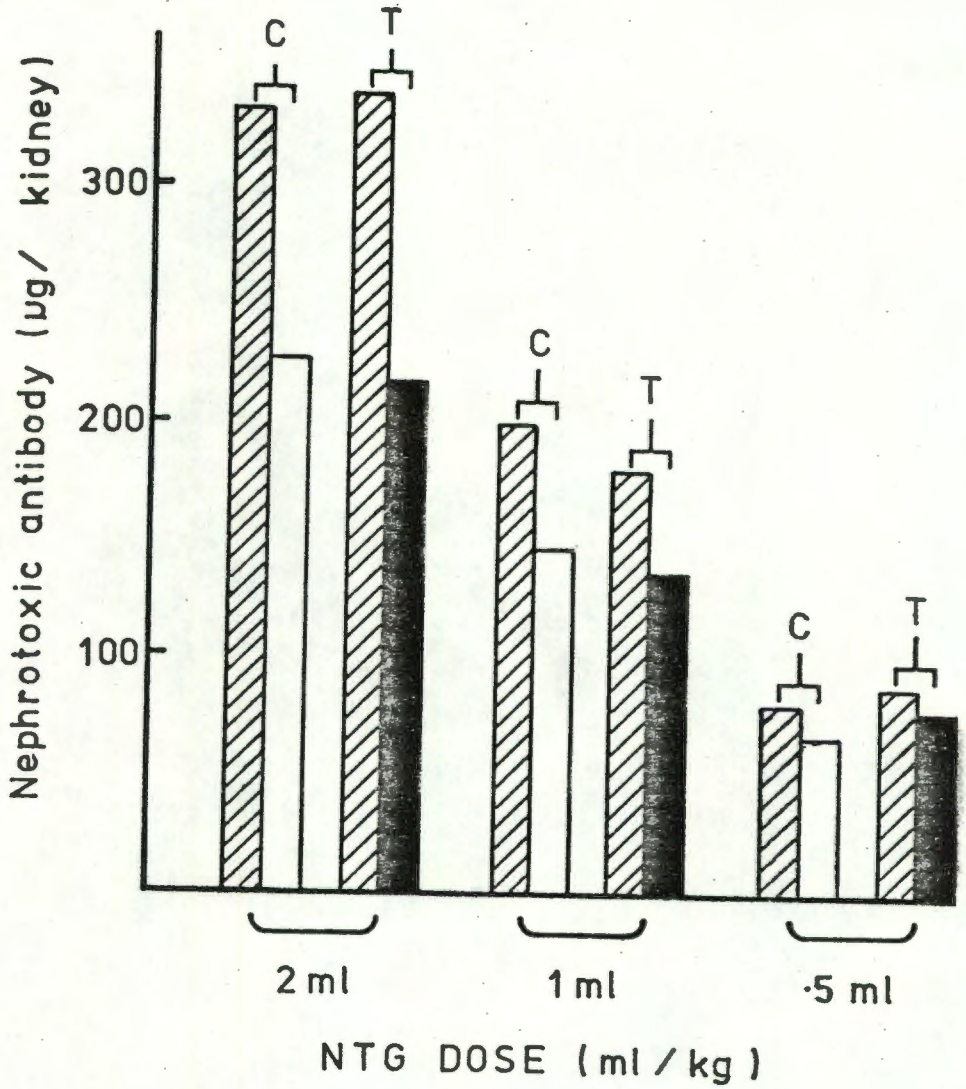


Fig.II.7

Nephrotoxic antibody bound on day 1, (▨) compared to 5 days after infusion of NTG (■,□) NTG given at the following doses; 2 ml/kg, 1 ml/kg, and 0.5 ml/kg b.wt. Test rabbits received CVF on day 1, (T), compared to controls (C)There was no significant difference.

It therefore appears that variations in the level of C3 as might be expected during NTN and following an acute phase stimulus, do not affect the amount of GBM bound NTAb at the onset of the autologous phase.

II.3.4 C-Reactive Protein Responses during the Heterologous Phase.

The response of C-reactive Protein (CRP) to the injection of a standard dose of NTG was assessed in a group of 21 rabbits which had been given 0.5 ml NTG/kg body weight, a dose insufficient to produce proteinuria during the heterologous phase, but still large enough to provoke an acute phase response.

The CRP response was highly variable. (Fig.II.6 and Table II.3) In general rabbits manifesting the greatest rise in CRP already had low detectable levels of CRP before the NTG was injected. The greatest rise was seen on day 1 and most levels returned to normal by day 3. It is of interest to note that 8 of the 20 rabbits, showed no response in CRP whilst other rabbits attained levels of up to 90 mg/100ml.

Fig.II.8 /....

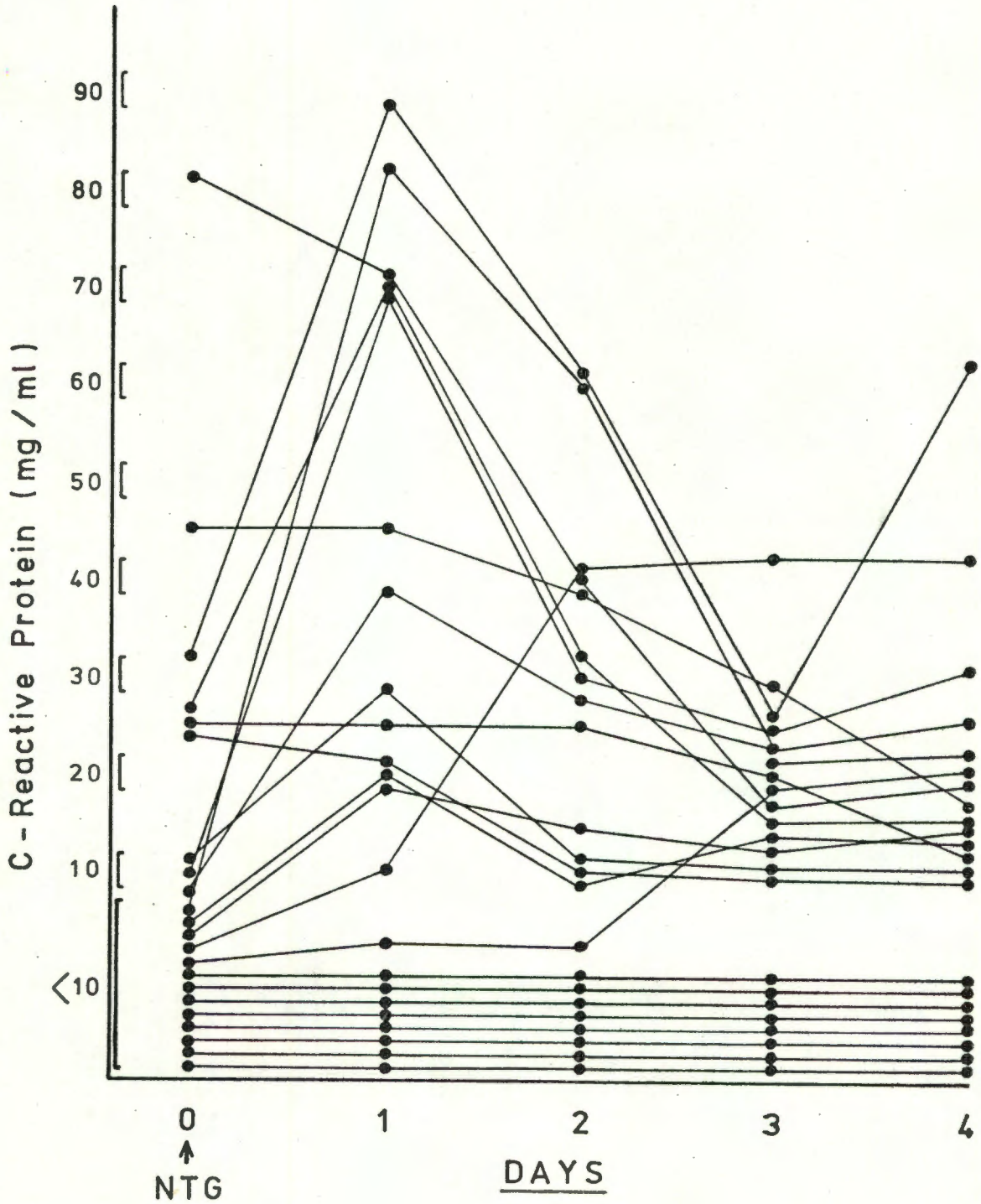


Fig.II.8

C-Reactive Protein responses following the injection of 0.5 ml/kg body weight NTG in 20 rabbits. Eight rabbits showed no response whatever, the maximum level obtained was 90 mg/100 ml

C-Reactive Protein (mg/ml)

Rabbit no.	Days following NTG				
	0	1	2	3	4
44	10	10	10	10	10
45	10	80	60	20	25
46	10	20	15	10	10
47	10	10	10	10	10
48	12	20	12	12	12
49	10	10	10	10	10
50	10	10	10	10	10
51	10	12	40	40	40
52	10	10	10	10	10
53	10	10	10	10	10
61	10	30	10	15	15
62	45	45	40	30	15
63	80	70	40	20	20
64	30	90	60	25	60
65	25	25	25	20	10
66	25	70	30	10	15
67	10	70	30	25	30
68	10	10	10	10	10
69	10	10	10	10	10
70	25	20	10	15	20
71	10	10	10	20	20

Table II.3

C-Reactive protein responses following the injection of NTG 0.5 mg/kg body weight.

As it has been suggested that the capacity to produce high titres of antibody may be related in some way to the magnitude of the CRP response after injury (Wood 1953a,b), the maximum level of CRP attained in each rabbit following on the injection of the NTG was compared with the severity of the ensuing autologous phase. Rabbits were divided into two groups: A, with minimal autologous phase injury and B, with severe injury (Fig. II.9). See Part V for full details regarding grouping of these animals.

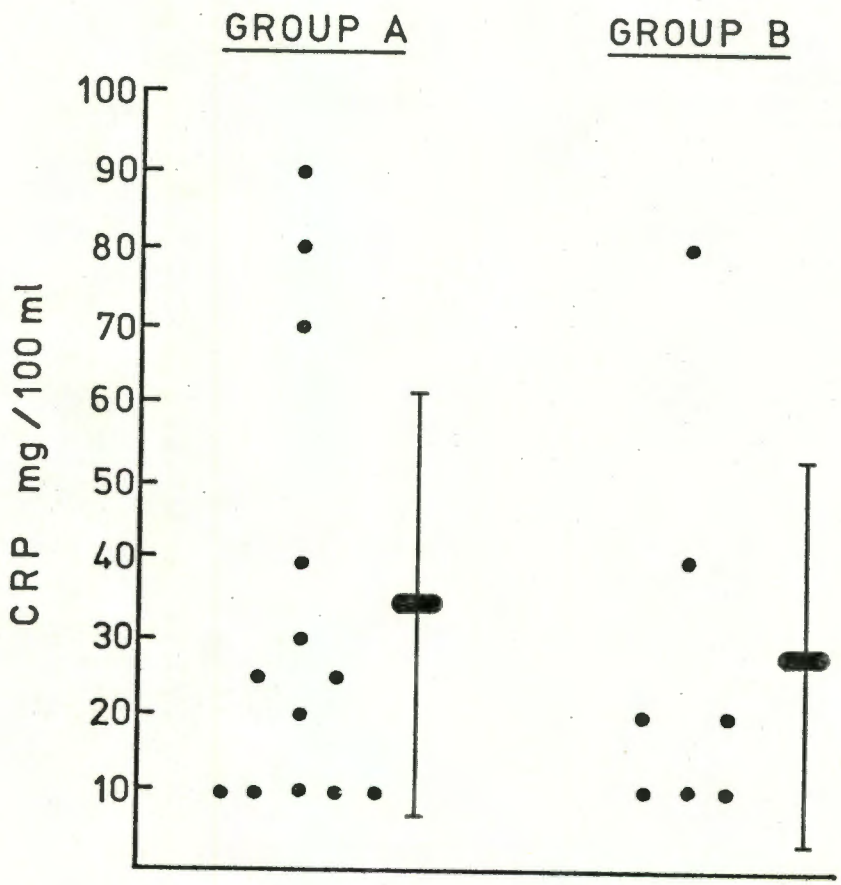


Fig.II.9

Maximum CRP levels attained during the heterologous phase of NTN by rabbits of Group A (n=10), compared to Group B (n=7). The difference is not significant ($p > 0.5$)

As can be seen from Fig II.7 the capacity to produce high levels of CRP did not correlate with the severity of the ensuing autologous phase. The central issue however still remains, that is, whether CRP, acting as an inflammatory mediator, might potentiate injury. This issue is considered further in Parts III & IV.

II.3.5 Changes in level of C3

Depressed levels of C3 were noted within 6 hours in the rabbits receiving a NTG dose of 1.5 ml/kg body weight, but were well preserved in those receiving smaller doses. Levels returned to normal or above normal levels, by day 2 (Fig.II.8, table II.4). The decline appeared to be related to the degree of proteinuria, however it is probably due to "immune activity", rather than urinary loss, as shown by Thomson (1975).

Fig.II.10 /...

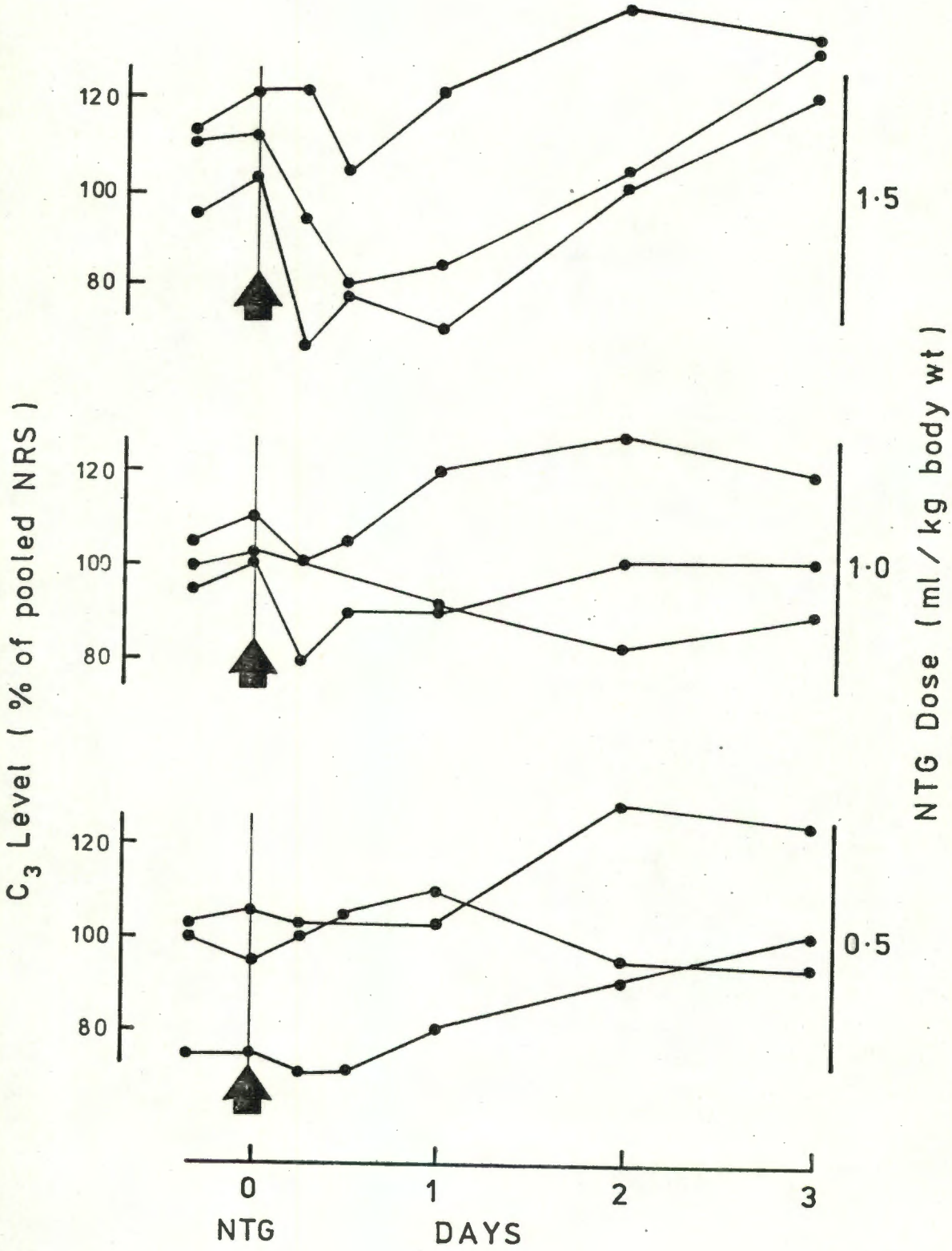


Fig.II.10

Changes in C3 level (percentage of pooled normal rabbit serum) following the injection of NTG at 3 dose levels: 0.5 ml/kg, 1.0 ml/kg, and 1.5 ml/kg body weight.

NTG Dose ml/kg	C3 Level Days						
	-1	0	6 hrs	12 hrs	24 hrs	2	3
1.5	110	112	95	80	85	105	130
1.5	110	120	120	105	120	140	130
1.5	95	103	65	80	70	102	130
1.0	95	100	80	90	90	100	100
1.0	105	110	100	105	120	130	120
1.0	100	100	-	-	90	80	90
0.5	75	75	70	70	80	90	100
0.5	100	95	100	105	110	95	95
0.5	100	105	100	-	100	130	125

Table II.4

Changes in C3 level (percentage of pooled normal rabbit serum) following the injection of NTG at 3 dose levels: 0.5 ml/kg, 1.0 ml/kg, and 1.5 ml/kg body weight.

II.3.6 Changes in level of fibrinogen

Following the administration of NTG, fibrinogen levels invariably rose, reaching a maximum by day 1 to 2 (Fig.II.11 and Table II.5). In contrast to C3, the magnitude of the change did not appear to correlate with the dose of NTG administered. In fact, rabbits which received the "lower dose" of NTG, appeared to have a higher rise in fibrinogen (mean rise of 32% sd 15) when compared to rabbits receiving a lower dose (mean rise 18% sd 10.4).

Fig.II.11/...

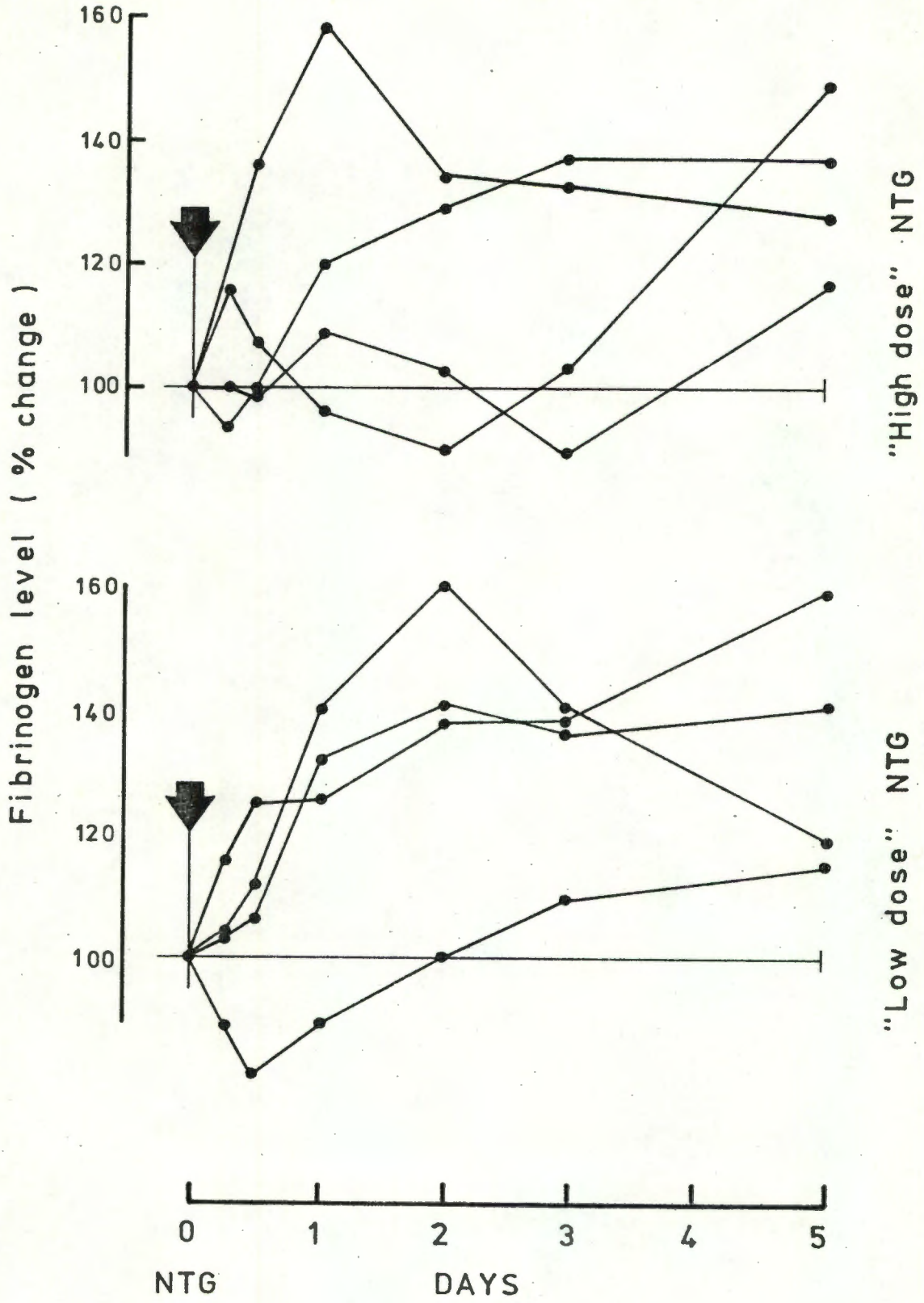


Fig.II.11

Alteration in fibrinogen levels following the injection of NTG. Two groups of four animals. One group received low dose NTG (0.25 - 0.5 ml/kg) the other high dose NTG (1.0 - 1.5 ml/kg body weight)

Dose of NTG ml/kg	Fibrinogen level % change						
	0 hrs	6 hrs	12 hrs	24 hrs	2	3	5
1.0 to 1.5	100	-	135	160	135	130	125
	100	115	110	95	90	105	130
	100	100	95	110	105	90	120
	100	95	100	120	130	140	140
0.25 to 0.5	100	90	82	90	100	110	118
	100	118	125	128	140	142	160
	100	105	110	130	140	138	142
	100	108	110	140	160	140	120

Table.II.5

Alterations in level of fibrinogen following the injection of NTG at two dose levels; "Low dose" group received 0.25 - 0.5 ml/kg NTG, whilst the "High dose" group received NTG 1.0 - 1.5 ml/kg body weight.

II.3.7 Changes in renal function

The dose of NTG used to induce the heterologous phase 1.0 ml/kg body weight or less, was insufficient to produce any detectable impairment of renal function in any of the rabbits studied.

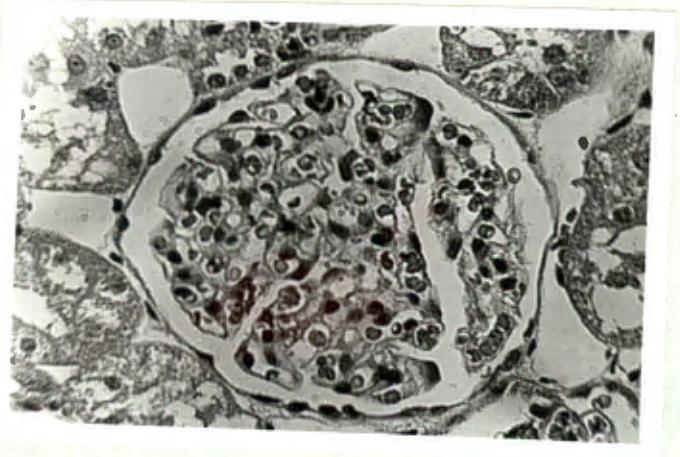
II.3.8 Light Microscopy

Renal tissue for light microscopy was taken 24 and 48 hours after injection of NTG.

Animals receiving small doses of NTG showed essentially normal histology, (P. I), whilst those receiving large doses (> 2 ml/kg or more) showed an infiltrate of polymorphonuclear leucocytes (P. 2).

(Normal rabbit kidney was prepared for purposes of comparison for light, immunofluorescent and electron microscopy.)

Photo 1 A normal rabbit glomerulus



LIGHT MICROSCOPY - HETEROLOGOUS PHASE

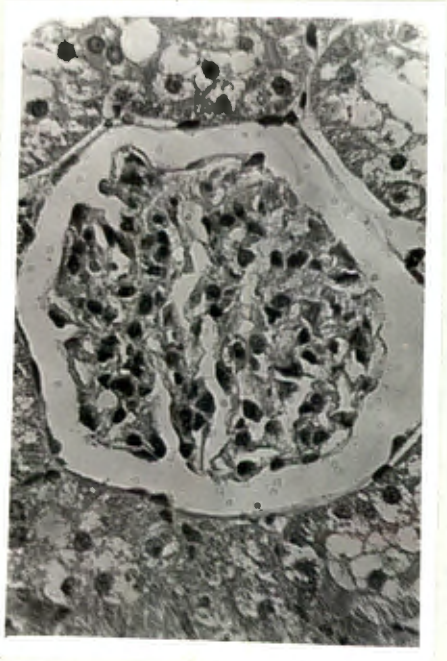
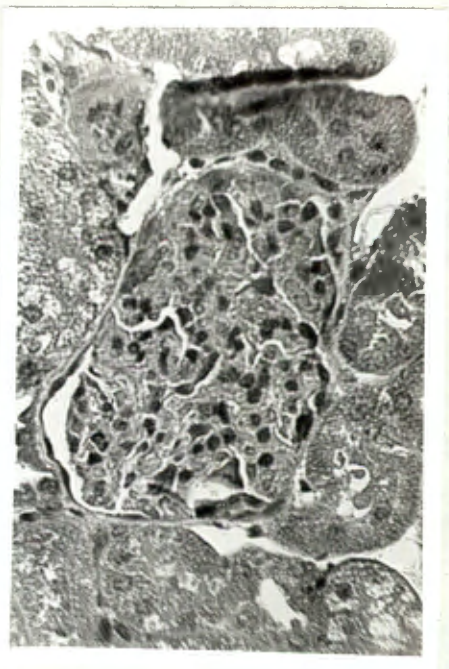


Photo 2

Day 1 Mild hypercellularity with occasional polymorphs.

Photo 3

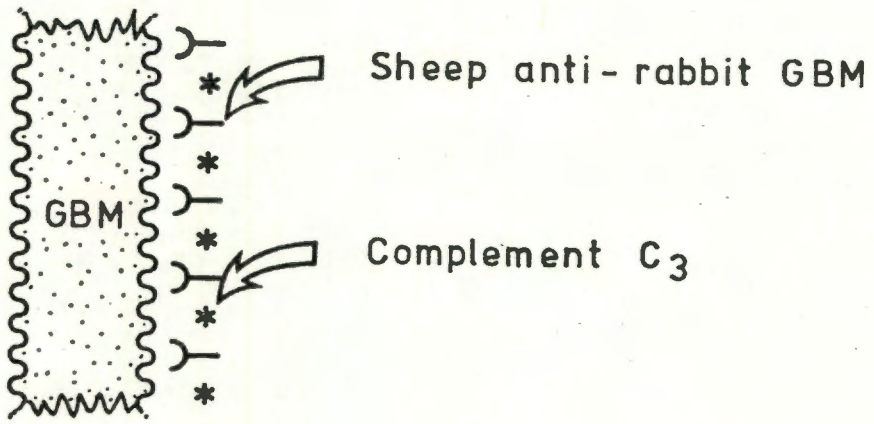
Day 1 Same features as photo 2 but capillary loops not as patent.



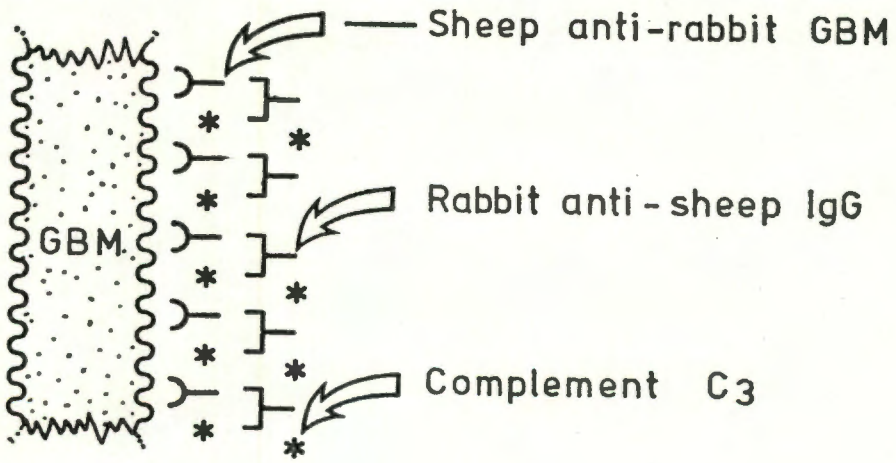
II.3.9 Immunofluorescent microscopy

The antibodies which can be demonstrated by immunofluorescent microscopy during the two phases of NTN are shown in the diagram below.

HETEROLOGOUS PHASE



AUTOLOGOUS PHASE



Heterologous phase

Strong linear staining for sheep IgG (photo 4) and rabbit

C3 (photo 5) was noted within 6 hours of NTG administration in all kidneys studied irrespective of the dose of NTG given. Staining for rabbit IgG was negative , as were all stains applied to tubules and vessels.

HETEROLOGOUS PHASE

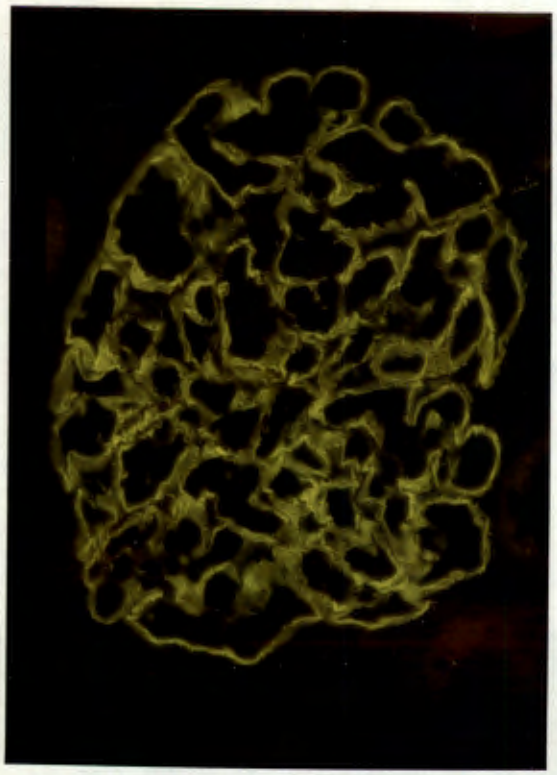


Photo 4

Sheep IgG, Day 1.

Negative staining of tubules and tubular basement membrane.

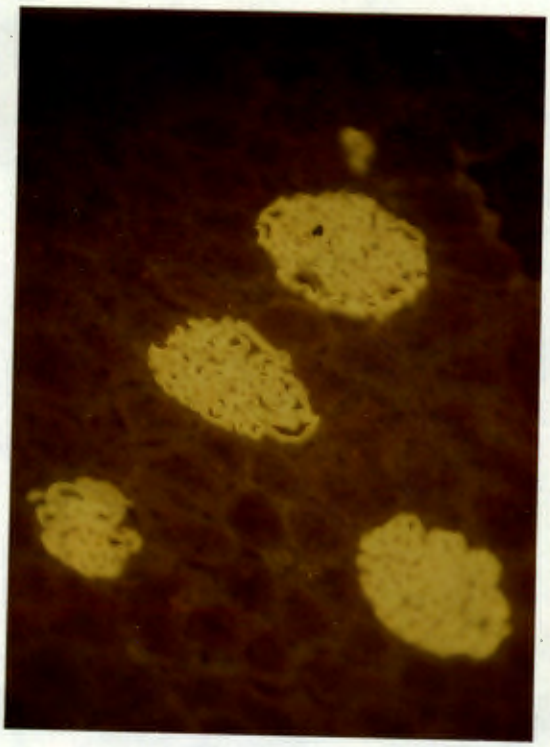
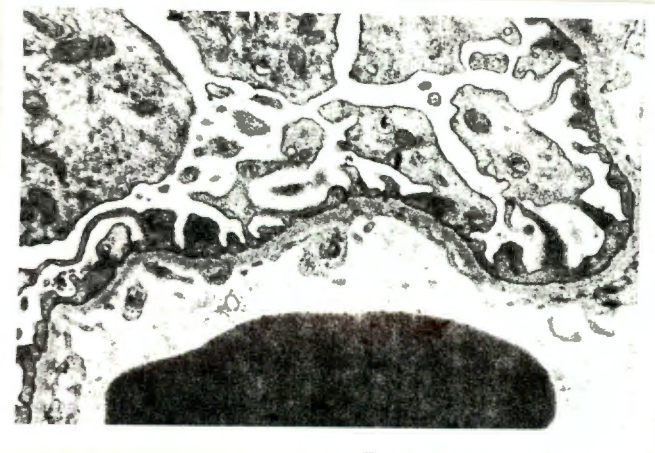


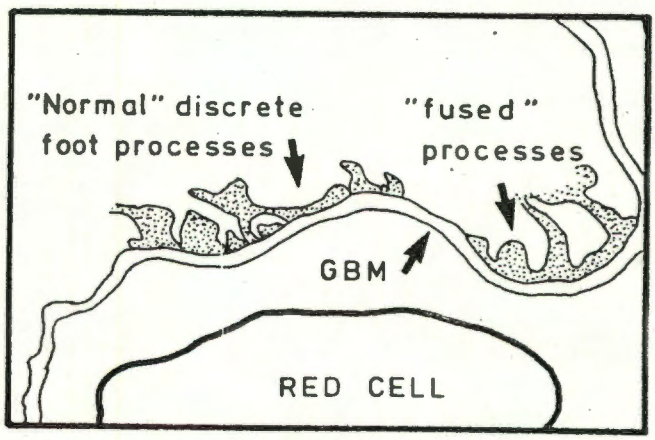
Photo 5

Rabbit C3, day 1.

II.3.10 Electron microscopy

Only minimal changes in morphology were noted, consisting of mild fusion of epithelial foot processes, with no evidence of immune deposits. (Photo 6)





II.4.0 RESULTS - AUTOLOGOUS PHASE

II.4.1 Dose response curve

Initially a dose of 0.125 ml/kg to 1.0 ml/kg b.wt was used to establish the appropriate dose of NTG to be used in later experiments. In general, the higher the dose of NTG, the greater the likelihood that the animal would develop autologous phase injury. It was also likely to be more severe and longer lasting. Typical autologous phase responses at various doses of NTG are illustrated in Fig.II.12

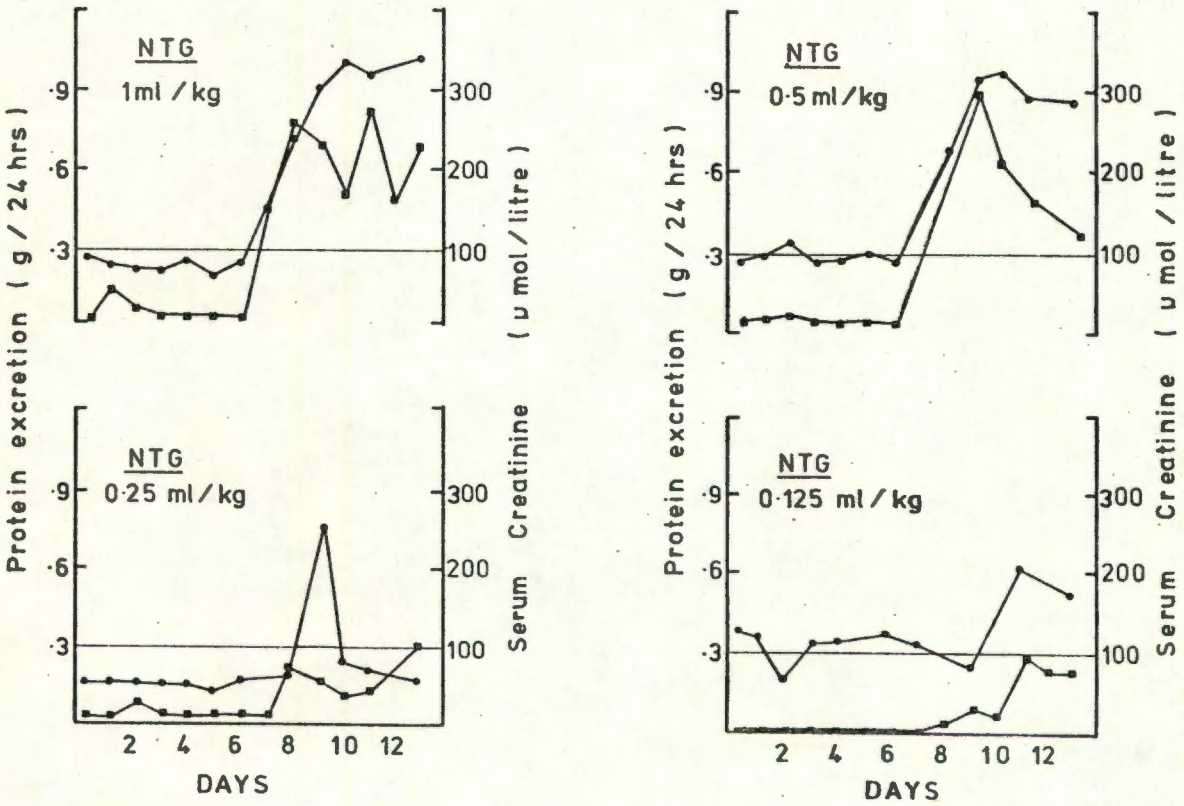


Fig.II.12

Autologous phase nephritis in rabbits given various doses of NTG (0.25 ml/kg, 0.5 ml/kg, 1.0 ml/kg, 2.0 ml/kg body weight) Serum creatinine (●) and 24 hour protein excretion (□)

The injury caused by the NTG during the autologous phase was characterised by extreme variability. At a dose of 0.5 ml/kg b.wt, chosen to show up differences in response between individual rabbits, about 60 % of rabbits developed overt disease, whilst the remainder remained reasonably "well", this is discussed further in Part V.

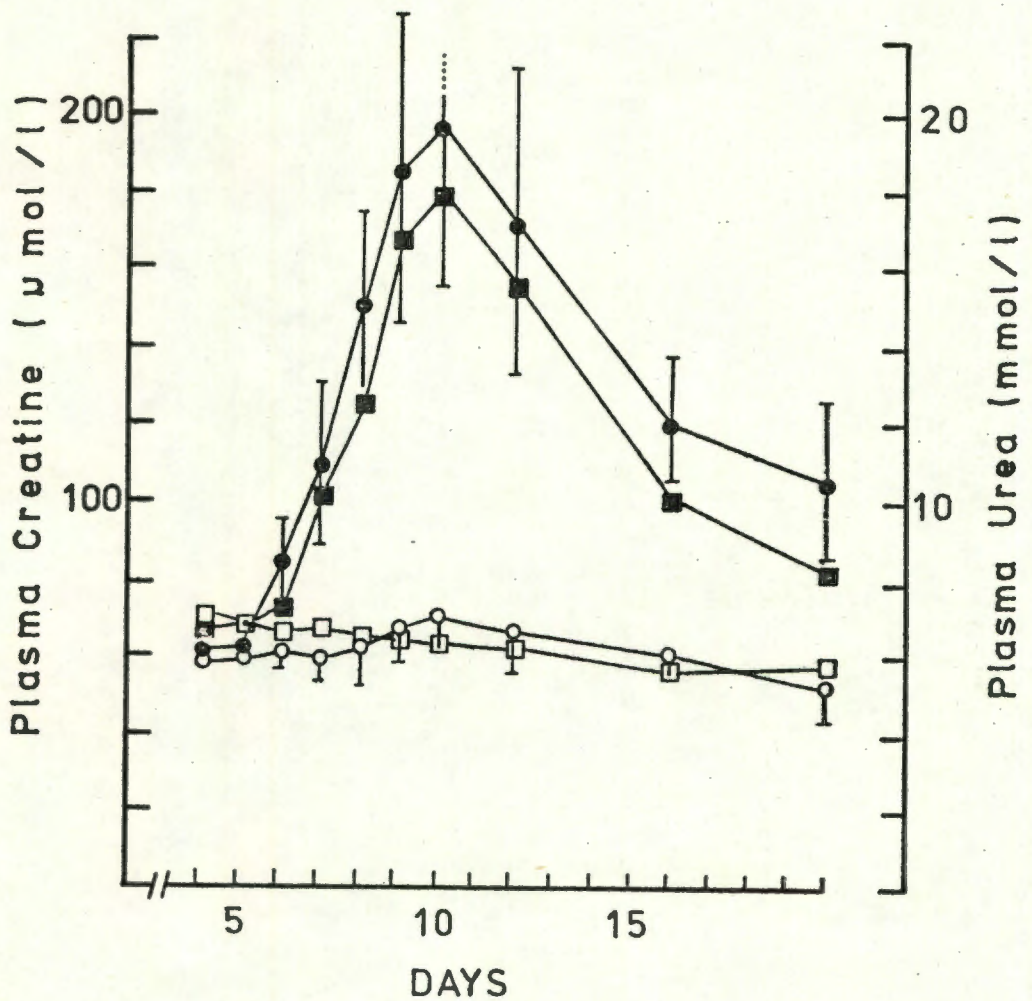


Fig.II.13

Serum creatinine (□) and urea (○) levels (mean ± SE), during the autologous phase in 22 rabbits, all having received the same dose of NTG (0.5 ml/kg body weight). Rabbits were grouped into those with (■, ●) and those without (□,○) overt disease. (These responses are analysed in detail in Part V) II.4.2

II.4.2 Development of immune complexes

Although the primary mechanism of injury during the autologous phase is considered to result from the binding of autologous antibody, circulating immune complexes also form, as is illustrated in Figure II.14.

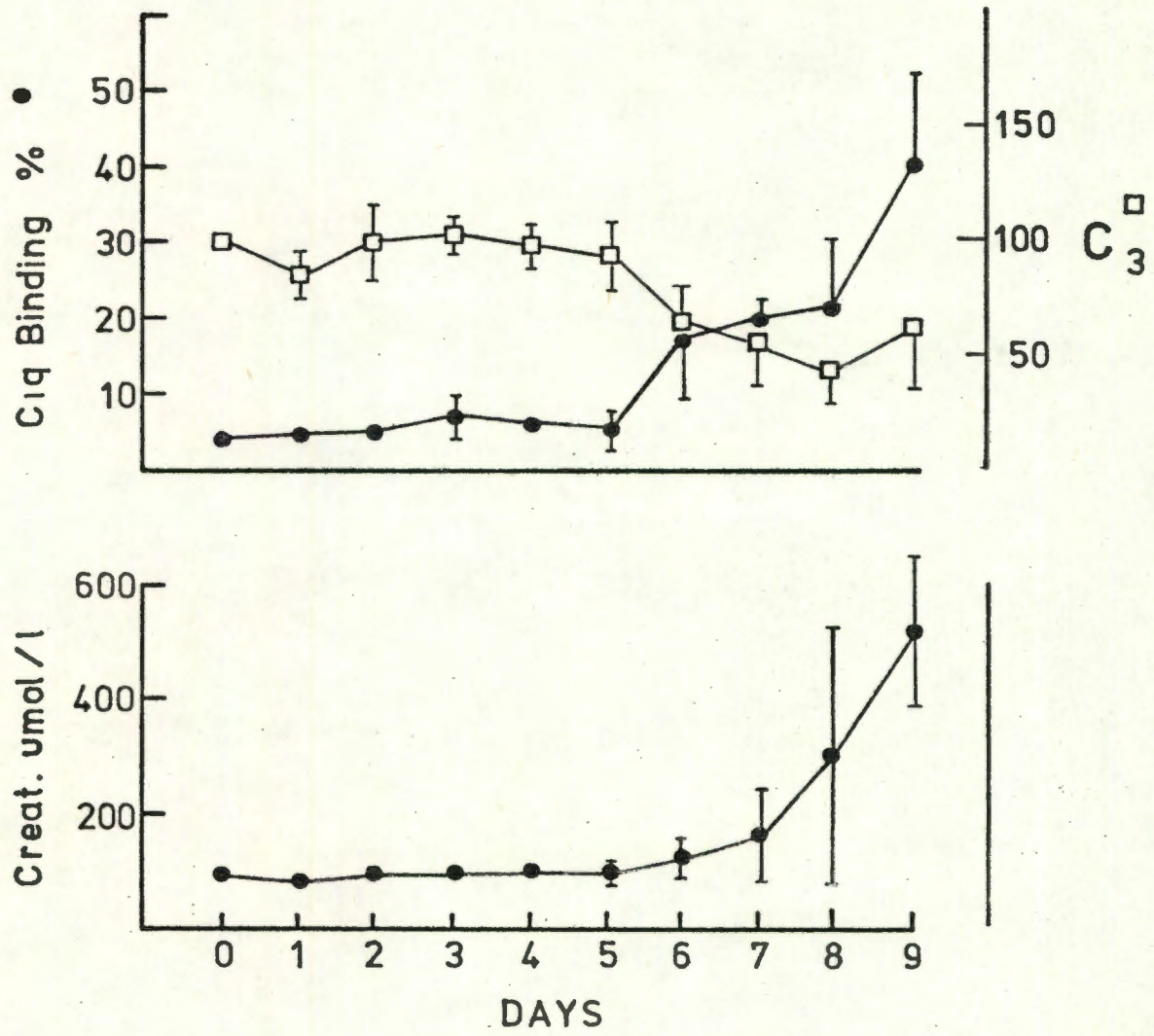


Fig.II.14

Immune complex formation and changes in level of C3 during the autologous phase. C1q binding % (●) and C3 level % change (□). The rising creatinine indicate the start of the autologous phase.

These immune complexes did not seem to play a major role as the administration of extra sheep globulin at the onset of the autologous phase, a technique which should enhance the formation of complexes, did not appear to worsen injury. Similarly immune deposits were not a prominent feature on electron microscopy, early in the autologous phase. The decline in level of C3 reflected "immune" activity with C3 consumption.

II.4.3 C-Reactive protein response

The CRP response during the autologous phase is illustrated in Fig.II.15 and Table II.6. Animals developing severe disease (Group A, n=15) during the autologous phase had a significantly higher CRP response with a mean level on day 10 of 45 ug/ml (SD 26), when compared to those with mild or no disease (Group B, n=7), with a mean level on day 10 of 12 ug/ml (SD 4.5). The difference is significant. ($p < 0.0$)

Fig.II.15 /...

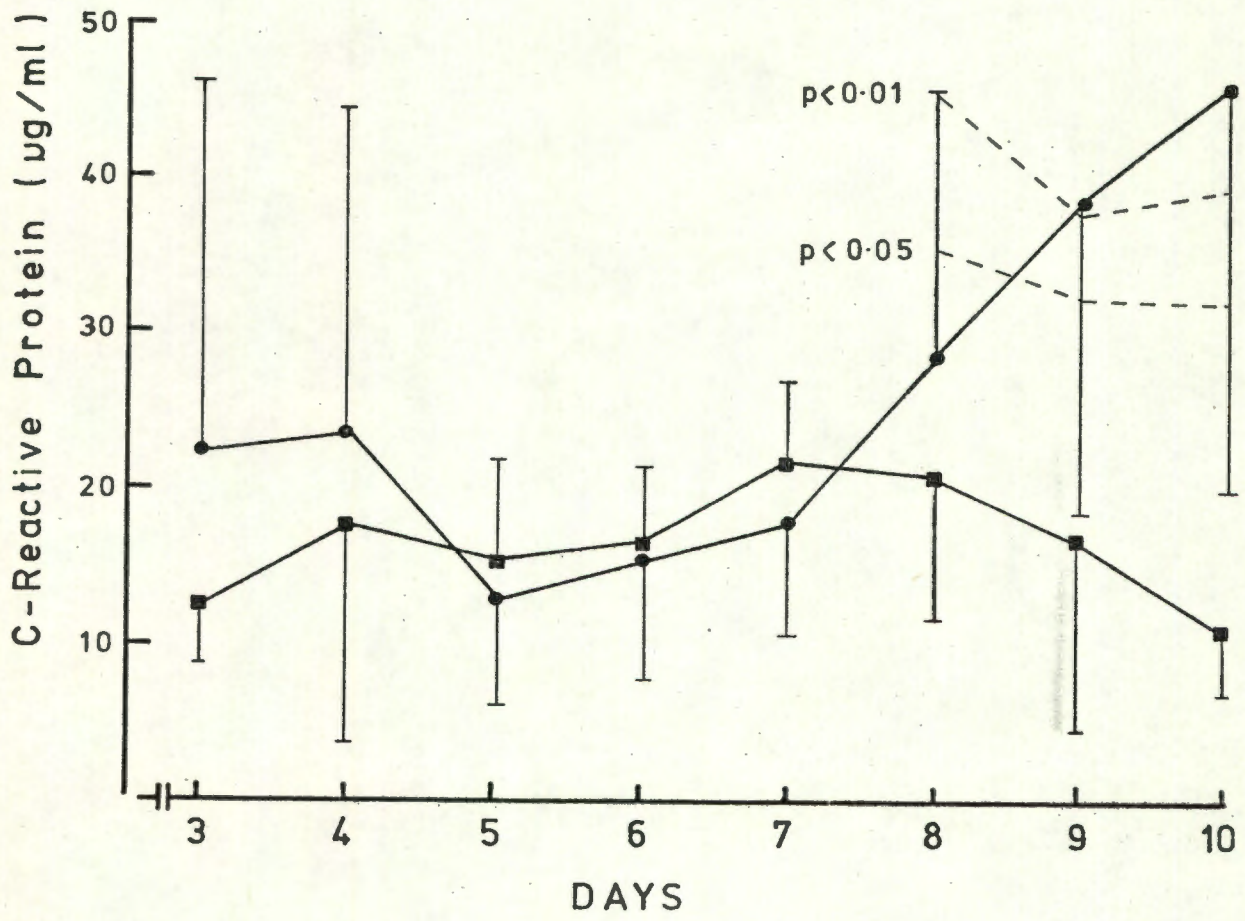


Fig.II.15

CRP response during the autologous phase. Animals with severe disease (●), compared to those with little or no disease. The difference is significant ($p < 0.01$). See Table II.6

GROUP A ANIMALS

Rabbit no.	Maximum creatinine umol/l	Day 3	4	5	6	7	8	9	10
		C-Reactive Protein ug/ml							
33	400	70	70	25	20	15	15	25	40
32	350	25	20	20	20	20	60	75	70
44	260	10	10	10	10	10	15	15	10
62	400	15	30	20	30	30	35	60	60
66	130	5	10	15	20	20	35	40	-
69	150	10	10	0	10	20	10	15	-
Mean		22	25	13	17	18	28	39	45
SD		24	23	7.5	8.1	7.5	18.8	24.4	26

GROUP B ANIMALS

Rabbit no.	Renal function "normal"	Day 3	4	5	6	7	8	9	10
		C-Reactive Protein ug/ml							
34		10	10	10	25	25	30	40	20
46		20	20	20	20	15	10	15	10
48		15	15	15	20	20	15	10	10
50		10	10	10	15	25	25	10	10
52		10	10	10	10	35	30	10	10
68		10	10	10	10	25	20	30	-
Mean		12	18	15	17	24	22	18	12
SD		4	14.6	7.6	5.6	6.2	8.1	12.2	4.5

Table II.6

CRP response during the autologous phase. Animals grouped into those with severe disease (Group "A") for comparison with those with mild or no disease (Group "B"). All animals received the same dose of NTG.

II.4.4 Light microscopy

The renal histology of animals with severe disease was characterised by hypercellularity, a polymorphonuclear leucocyte infiltrate and "crescents". However in some animals with severe disease clinically, there was marked fibrinoid necrosis of glomeruli by day 8 - 10, resulting in almost total destruction, without the production of crescents.

No crescents were seen in animals with mild or no disease, and the cellular infiltrate was variable but mild.

The variability in response is illustrated by the following photographs.

Photo 7 /...

LIGHT MICROSCOPY - AUTOLOGOUS PHASE

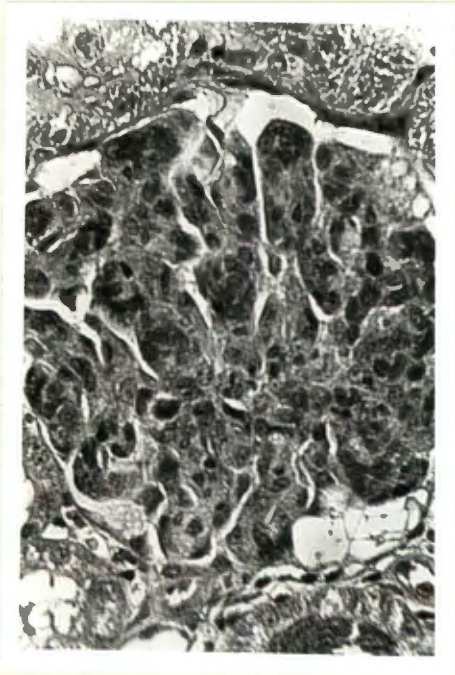


Photo 7

Early autologous phase Day 5

Showing - Hypercellularity, neutrophils, mesangial matrix increase, and compressed capillary loops.

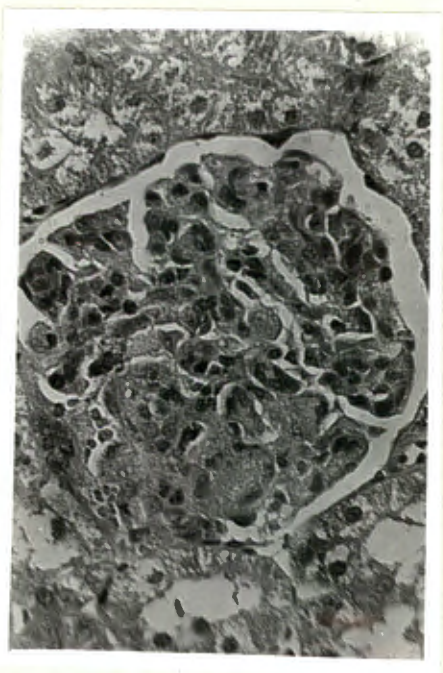


Photo 8

Early autologous phase

Day 5

Same as Photo 7, but showing fibrin present in capillary loops.

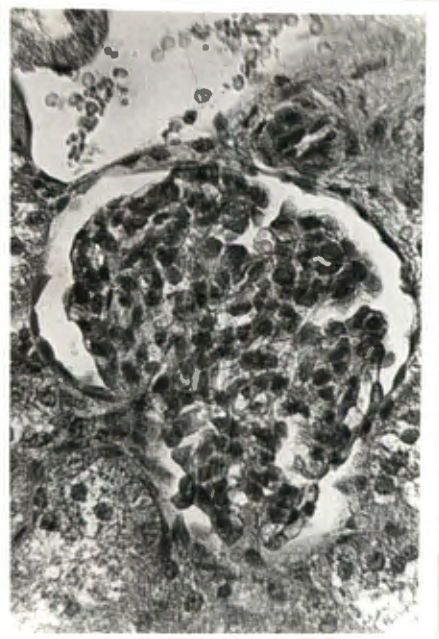


Photo 9

Autologous phase at maximum activity Day 12

Extensive hypercellularity involving mesangial, endothelial and epithelial cells with neutrophil infiltrate.

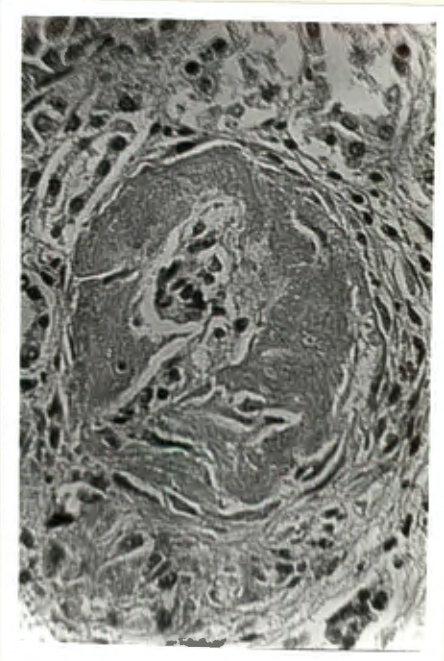


Photo 10

Autologous phase at maximum activity Day 13

Extensive fibrinoid necrosis with almost total destruction of the glomerulus.

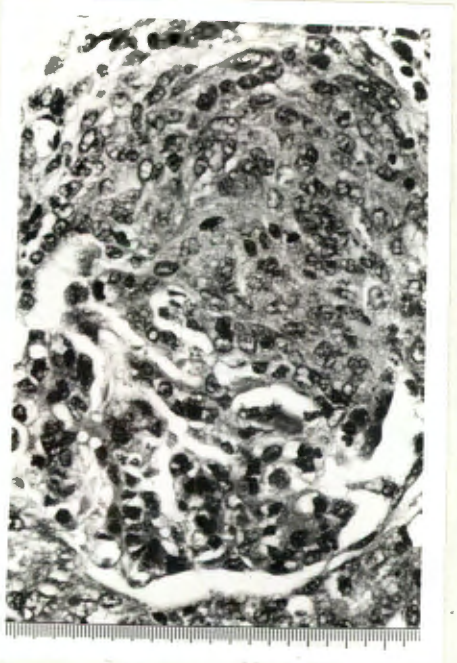


Photo 11

Late autologous phase Day 18

Crescent formation with hyper-cellularity of remaining tissue.

Photo 12

Late autologous phase.

This photograph illustrates a different response compared to that of photo 11 in that almost total destruction of the glomerulus has occured.

II.4.5 Immunoflourescent microscopy

Linear staining of C3, sheep anti-rabbit GBM and rabbit anti-sheep IgG were seen in all biopsies studied, staining was very strong in all instances irrespective of the severity of the disease. This was not an unexpected finding as the immunofluorescent technique employed is capable of detecting minute quantities of immunoglobulin.

IMMUNOFLUORESCSENT MICROSCOPY

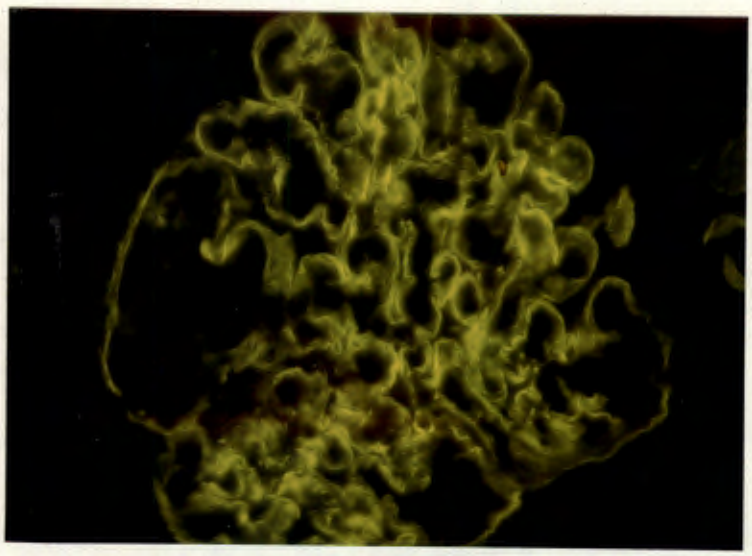


Photo 13

Linear staining of rabbit anti-sheep IgG.

Immunoglobulin is seen outlining the glomerular basement membrane. No staining of the tubules or interstitium.

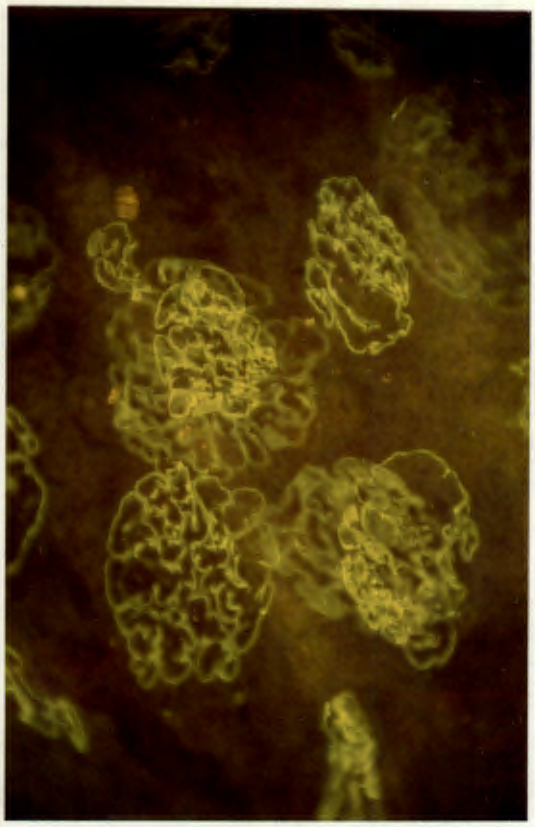
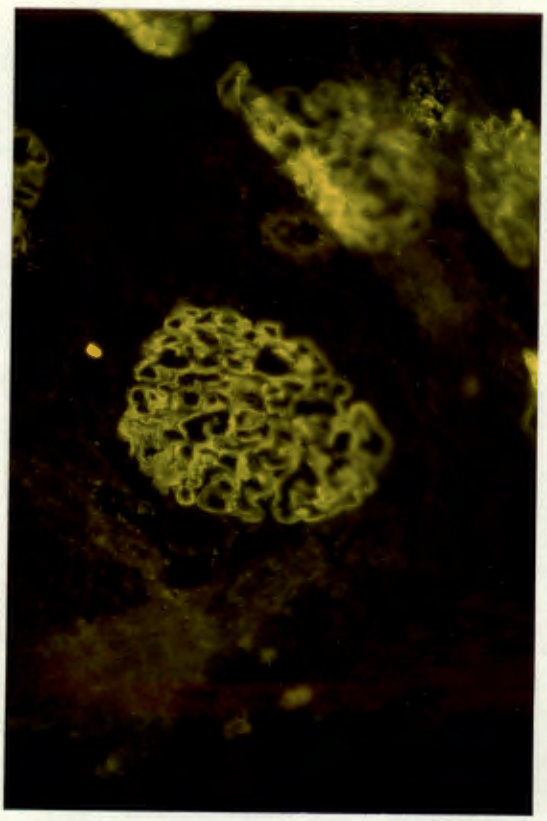


Photo 14

Immunofluorescence of sheep anti-rabbit GBM

Photo 15

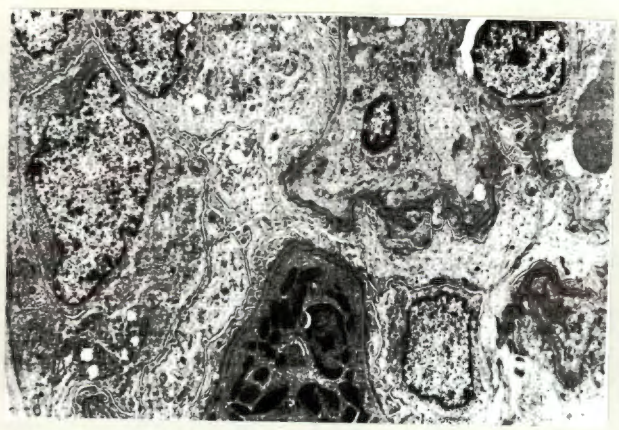
Immunofluorescence of rabbit C3.

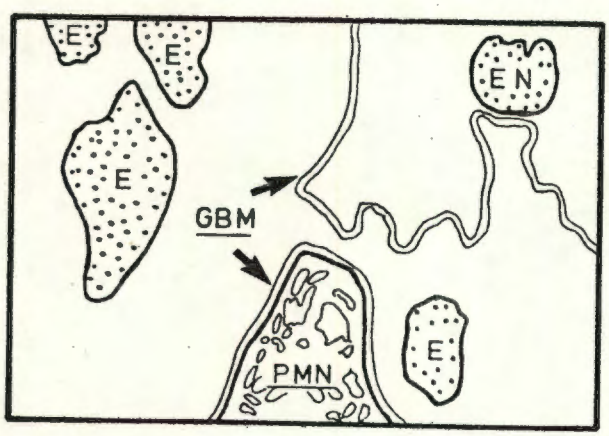


II.4.6 Electron microscopy

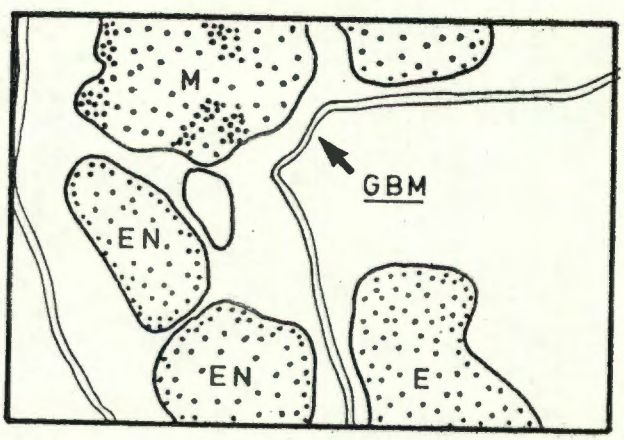
Great destruction was evident in most biopsies examined. The micrograph demonstrated here shows only two of the features, that is massive epithelial cell and macrophage proliferation in "crescents" and the relative absence of "immune deposits".

ELECTRON MICROSCOPY - AUTOLOGOUS PHASE





GBM - Glomerular basement membrane
PMN - Polymorphonuclear leucocyte
EN - Endothelial cell
E - Epithelial cell



GBM - Glomerular basement membrane
EN - Endothelial cell
E - Epithelial cell
M - Mesangial cell

PART III

THE EFFECT OF AN ACUTE PHASE STIMULUS ON NEPHROTOXIC NEPHRITIS

PART III

THE EFFECT OF AN ACUTE PHASE STIMULUS ON NEPHROTOXIC NEPHRITIS

III.0.0 INTRODUCTION

The type of glomerulonephritis associated with the presence of anti-GBM antibodies (Goodpasture's syndrome) is frequently aggravated by intercurrent infections. (As discussed in Part I.5.0)

The mechanisms whereby infections may enhance injury are varied (Part I.5.0). However, two main issues need to be considered. Firstly, the non-specific acute phase stimulus effect of infection might exert its influence through humoral, cellular or other mechanisms, to enhance injury. Alternatively, something inherent in the infection itself might be responsible. Immune complexes resulting from the infection may lodge in an already damaged glomerulus, or possibly, infection may cause specific enhancement of cellular inflammatory mediators.

The model of nephrotoxic nephritis (NTN) in rabbits was chosen to investigate this phenomenon further as both phases of NTN have certain features in common with the human counterpart. Heterologous anti-GBM antibodies are involved in the first phase of NTN whilst autologous antibodies directed to "planted" antigens (sheep IgG on

rabbit GBM), are involved in the second. Only the effect of an acute phase stimulus was studied although the model also lends itself to the study of the effect of infections.

III.1.0 INDUCTION OF AN ACUTE PHASE RESPONSE IN RABBITS

After a variety of preliminary experiments were done using various combinations of local irritants, I decided on a standard mixture consisting of 2% croton oil in liquid paraffin. In order to determine the optimal dose and the extent to which acute phase reactants would rise, the following experiment was done.

Doses of croton oil in liquid paraffin varying from 0.5 ml to 6 ml were injected subcutaneously into the flanks of 4 rabbits, with a further rabbit being used as a control to determine whether the mere handling, catheterization and bleeding of a rabbit would induce an acute phase response.

III.1.1 Fibrinogen response

Following the injection of the croton oil, there was a prompt rise in fibrinogen which was noticeable within 6 hours and attained a level of 200 to 300% of normal within 1 to 2 days, this is shown in figure III.16.

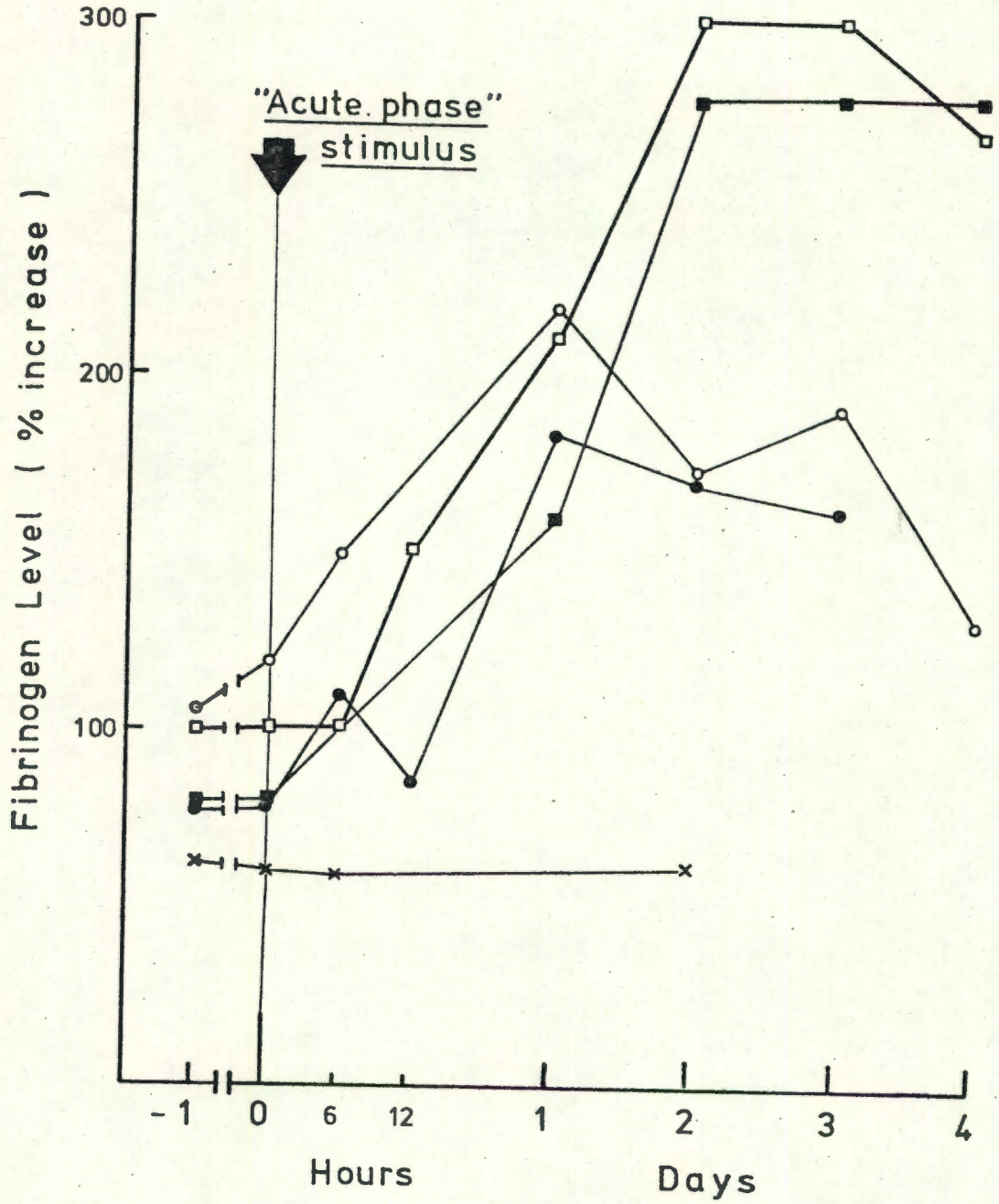


Fig.III.16

Fibrinogen response following the induction of an acute phase stimulus, (% increase). Croton oil in liquid paraffin given at following doses. 0.5 ml (●), 1.0 ml (o), 3.0 ml (■), and 6.0 ml (□). Control (x)

III.1.2 C-Reactive protein response

The rise in CRP was noted within 6 hours and reached a maximum by day 2 (Fig.III.17).

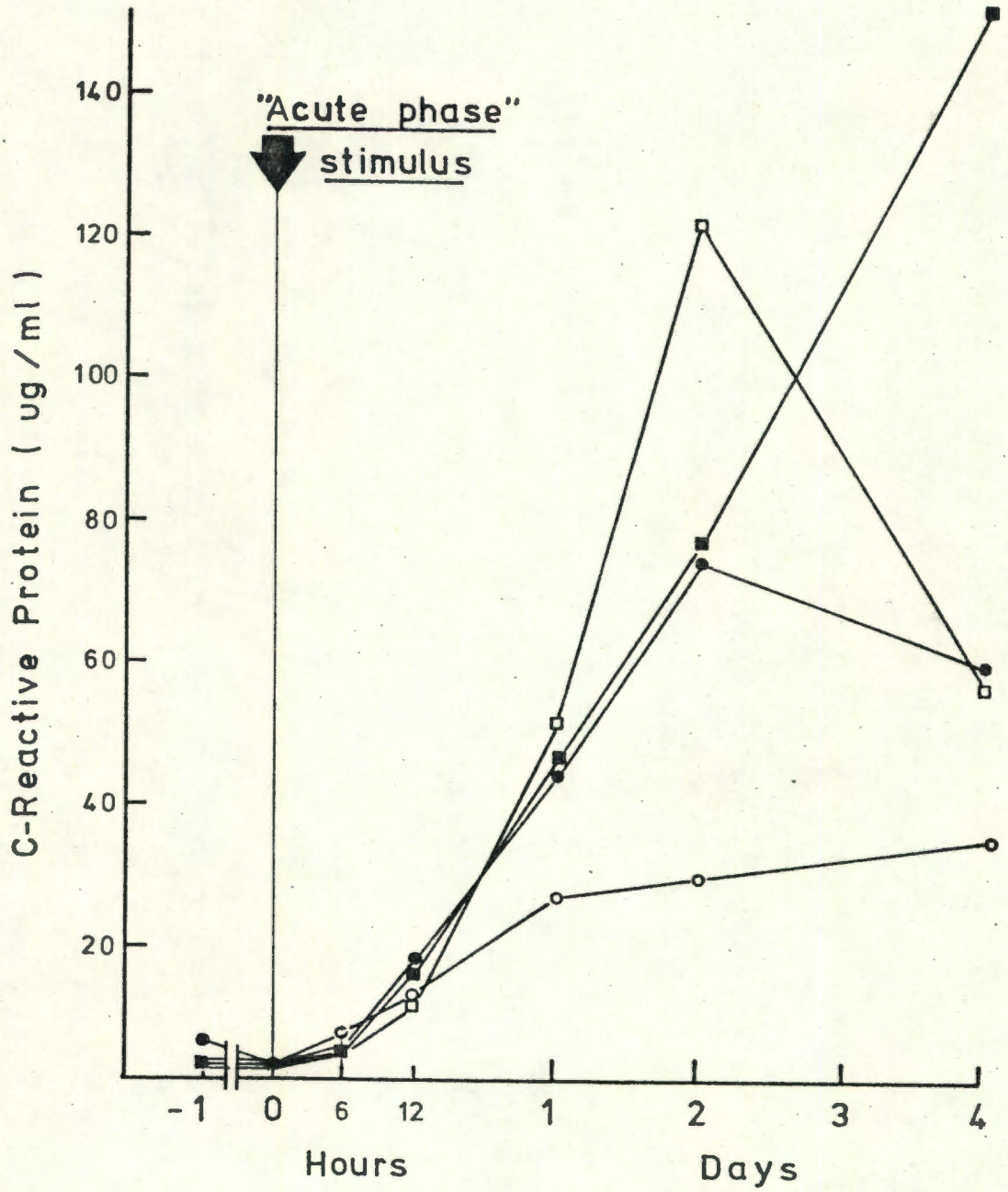


Fig.III.17
CRP response (ug/ml) following the injection of croton oil. Doses used; 0.5 ml (●); 1.0 ml (○); 3.0 ml (■); 6.0 ml (□).

III.1.3 C3 response

A definite rise in C3 only became apparent after one day but thereafter was sustained at approximately 150% of normal for four days (Fig. III.18).

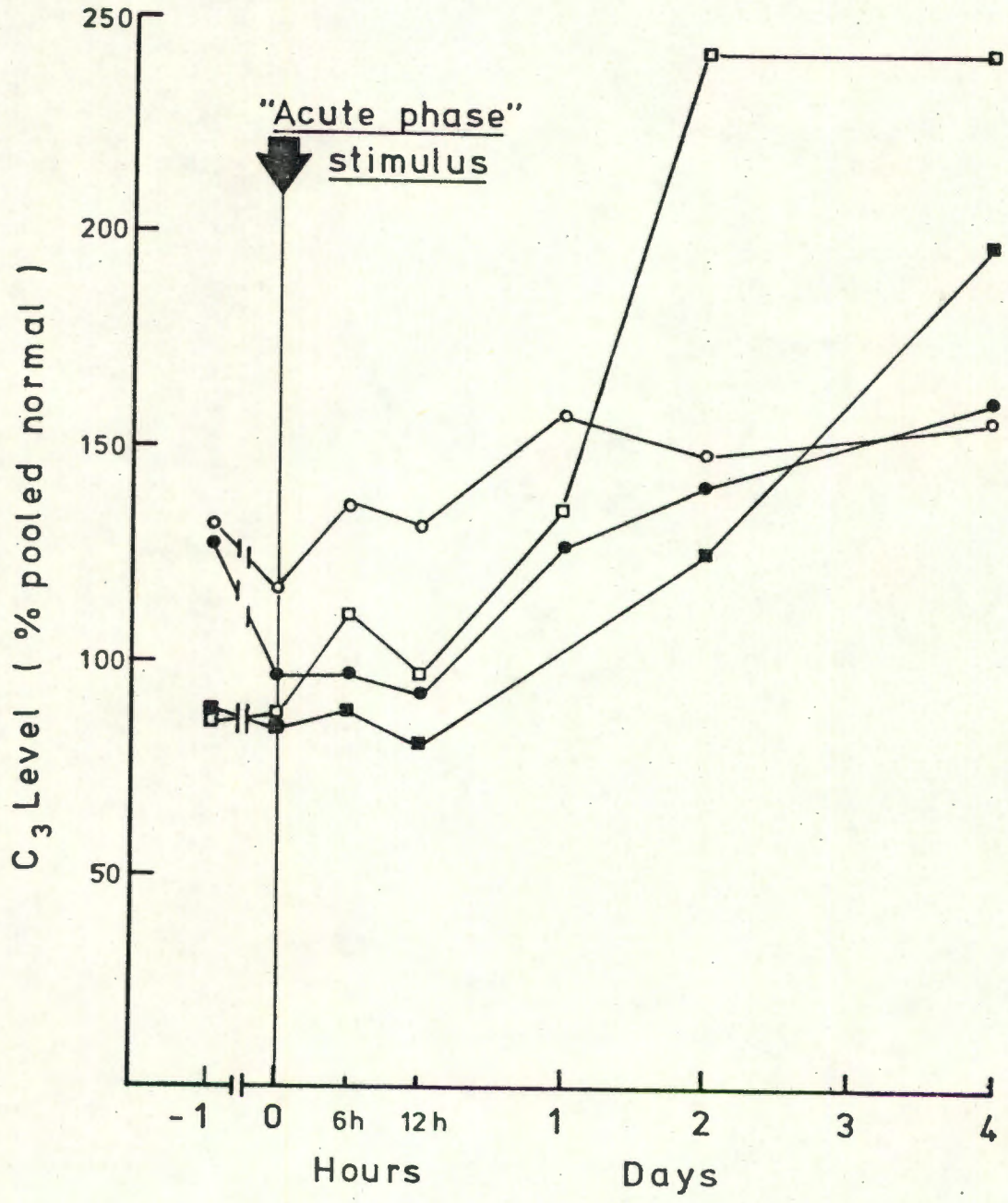


Fig.III.18

Change in level of C3 following the injection of croton oil. (% pooled normal rabbit serum). Dose levels 0.5 ml (●); 1.0 ml (○); 3.0 ml (■); 6.0ml (□).

III.1.4 Polymorphonuclear cell response (PMN)

All animals showed increased numbers of PMN's by 6 hours. However, by 12 hours the levels had virtually returned to normal. From day 1 onwards there was a gradually progressive rise in count, reaching approximately double the starting counts by day 4 (Fig. III.19).

See page (159) /...

III.1.5 Effect on proteinuria and renal function

None of the animals developed significant proteinuria or had a decline in renal function following the acute phase stimulus.

Controls

Neither of the two control rabbits which were handled, catheterized and bled showed any alteration in level of acute phase reactants whatever.

Fig.III.19 /...

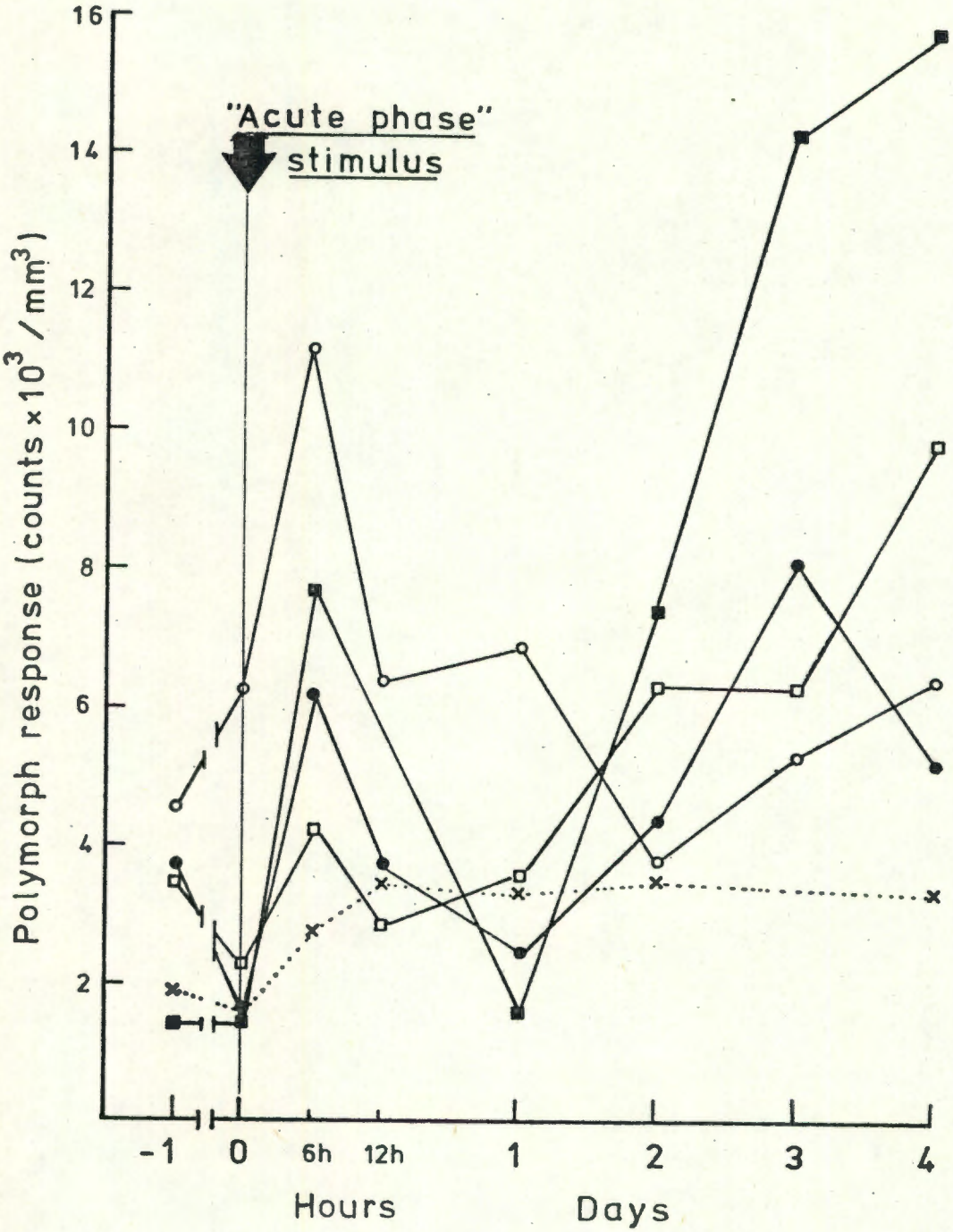


Fig.III.19

Polymorphonuclear leucocyte response following an acute phase stimulus. Dose of croton oil; 0.5 ml (●); 1.0 ml (○); 3.0 ml (■); 6.0 ml (□). Control rabbits were only handled, bled and catheterised (×).

III.1.6 Induction of an acute phase response during nephrotoxic nephritis

The previous experiments indicated that the injection of NTG, the autologous phase and the injection of croton oil would all result in an acute phase response. As a preliminary to Part III.2.0, the response to an acute phase stimulus during NTN, induced by the the injection of 2 ml/kg b.wt. of NTG, was determined.

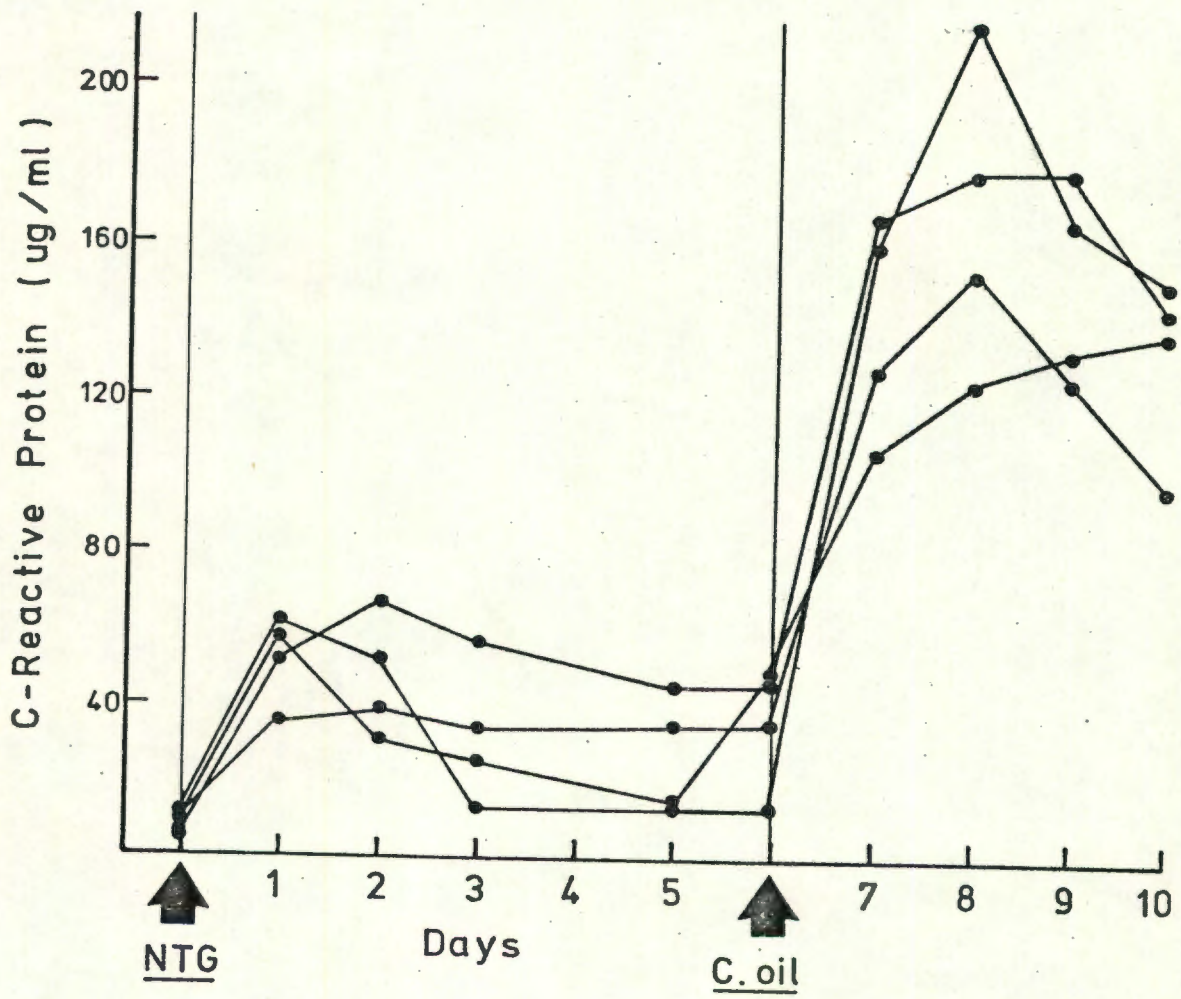


Fig.III.20

The effect of croton oil administration during nephrotoxic nephritis. (2 ml subcutaneously on day 6.)

It was apparent that the injection of croton oil produced a far more intense CRP response than the heterologous phase, even when a relatively large dose of NTG was used. The mean rise following NTG was 55 ug/ml and following croton oil, 170 ug/ml.

Comparison of acute phase responses encountered during NTN experiments

The relative effects of the induced acute phase response, the heterologous phase and the autologous phase as stimuli of an acute phase response are compared in figure III.18. The heterologous phase was induced by a relatively small dose of NTG, 0.5 ml/kg body weight (used for all experiments reported in Part V).

(a) The acute phase stimulus, produced a mean rise in CRP of 160 ug/ml; an increase in fibrinogen of 285 %; and of C3, of 183 %.

(b) The heterologous phase produced a rise in CRP of 25 ug/ml; a rise in fibrinogen of 80 %; and in C3 of 50 %.

(c) The autologous phase, produced a rise in CRP of 80 ug/ml. Fibrinogen and C3 levels were not measured in this experiment.

Fig.III.21 /...

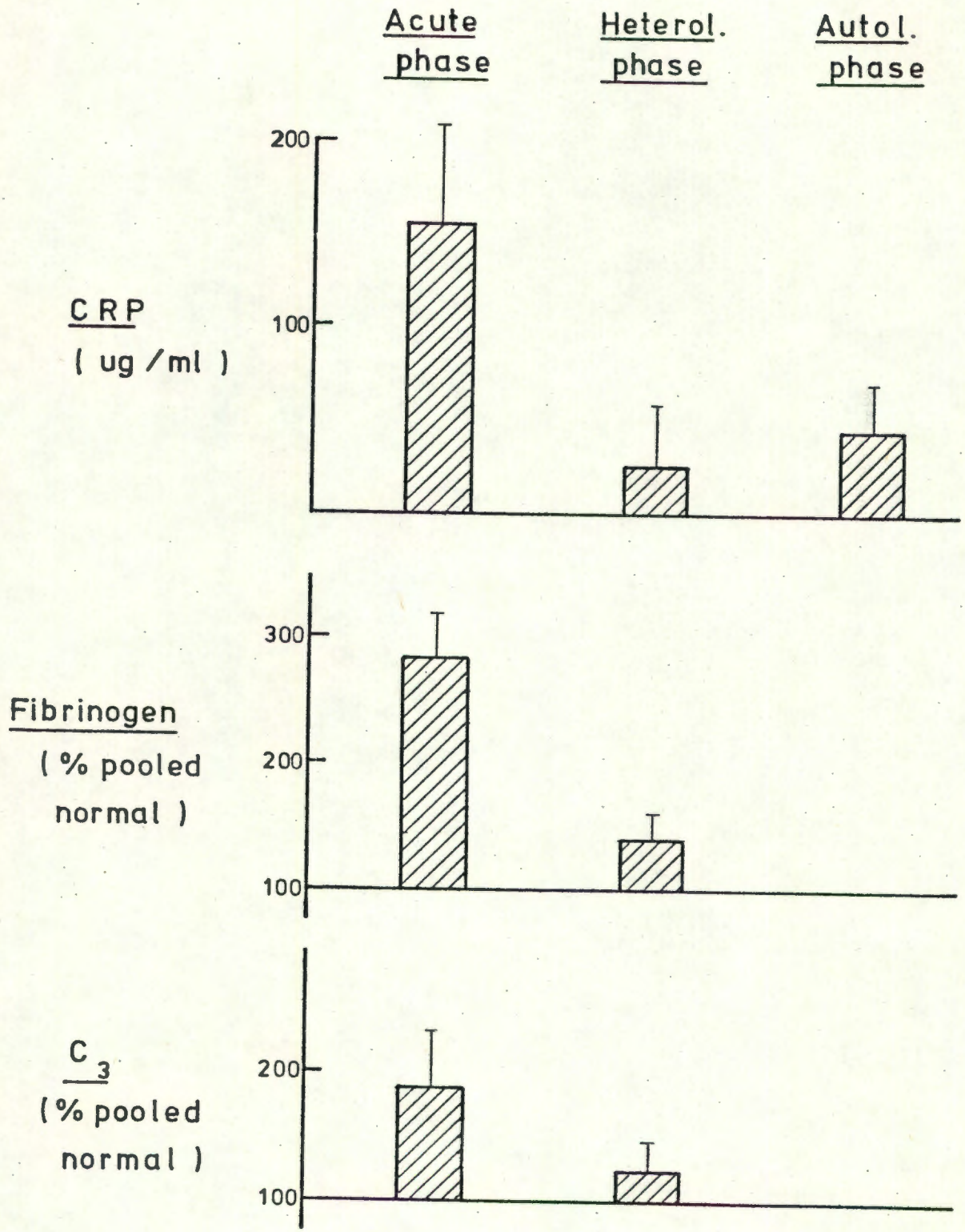


Fig.III.21

The effect of an acute phase stimulus, the heterologous and autologous phases on CRP, Fibrinogen, and C3. (Mean ± SD)

III.1.7 Conclusions

These experiments demonstrated that an acute phase response could reliably be induced in rabbits by the injection of croton oil in liquid paraffin and that a dose of 2 ml of a 2% solution appeared optimal. This was therefore used in all subsequent experiments as the stimulating dose.

The magnitude of the response as assessed by CRP, fibrinogen and C3 levels was much greater in all instances than the acute phase response resulting from either the heterologous or autologous phase of NTN.

An acute phase response by itself did not appear to cause any impairment of function in normal rabbits.

III.2.0 THE EFFECT OF AN ACUTE PHASE STIMULUS ON THE HETEROLOGOUS PHASE OF NEPHROTOXIC NEPHRITIS

The effect of an acute phase stimulus on the heterologous phase of NTN was assessed by inducing such a response prior to the administration of the nephrotoxic globulin (NTG). The effect on the subsequent 24 hour protein excretion, creatine and urea was then determined.

III.2.1 Experimental protocol

The rabbits were grouped as follows:

Group 1 (n = 6) received "high dose" NTG at 2 ml/kg body weight.

Group 2 (n = 8) received "low dose" NTG at 1 ml/kg body weight.

Each group consisted of the test rabbits which received 2 ml of 1% croton oil in liquid paraffin subcutaneously in the flank 24 hours prior to injection of NTG. The control rabbits were normal healthy rabbits. The "test" and "control" rabbits, all males, were matched for weight. Before the start of the experiment, the urine of all the rabbits was screened to ensure that no rabbits had underlying renal disease.

Immediately before the injection of the NTG all rabbits were catheterized to ensure that their bladders were empty. All urine was collected in metabolic cages and at the end of 24 hours the rabbits were killed and again catheterized

to ensure complete collection of the 24 hour urine sample.

Urine protein, creatinine and urea were measured as described in Part II.1.18.

III.2.2 Results (Fig.III.22 and Table III.7)

(a) "High dose" NTG (2 ml/kg body weight)

The test animals had a mean protein excretion of 645 mg/24 hours (SEM 92) whilst the control animals passed 606 mg protein/24 hours (SEM 81). This difference was not significant.

(b) "Low dose" NTG (1 ml/kg body weight)

The test animals had a mean protein excretion of 148 mg/24 hours (SEM 37) and the controls 128 mg/24 hours (SEM 37). Again this difference was not significant.

Fig.III.22 /...

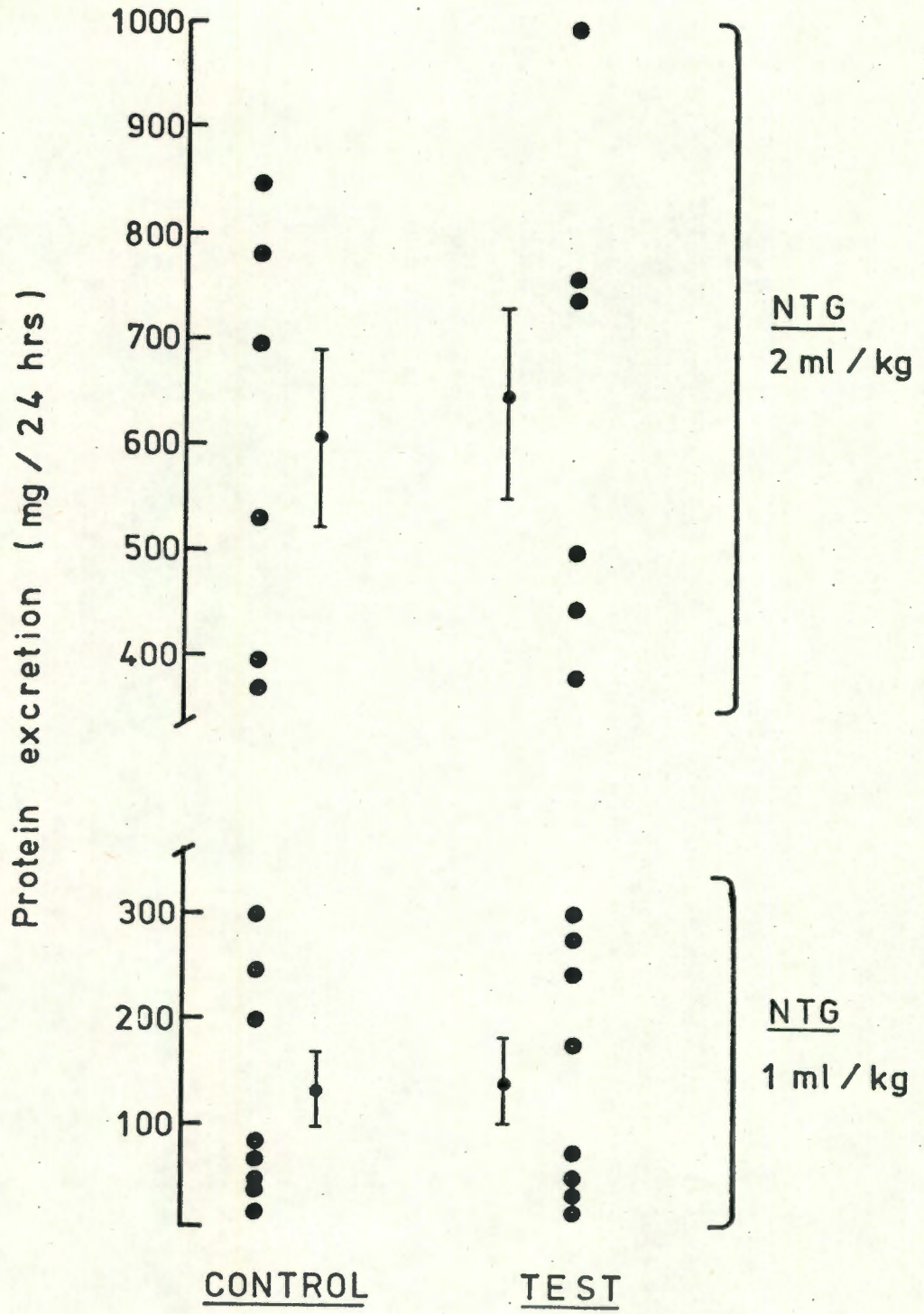


Fig.III.22

The effect of an acute phase stimulus on the Heterologous phase of Nephrotoxic nephritis. Test animals received croton oil 24 hrs before injection of NTG. (given at 1 ml/kg and 2 ml/kg body weight) The difference in protein excretion was not significant. (See text)

PROTEIN EXCRETION

mg/24 hours

	NTG 2 ml/kg		NTG 1 ml/kg	
	Control	Test	Control	Test
	850	1000	300	300
	780	750	250	280
	700	750	200	230
	530	500	90	180
	400	450	70	70
	380	420	50	50
			50	50
			20	30
Mean	606	645	128	148
SEM	81	92	37	39

Table III.7

24 Hour protein excretion during the heterologous phase of NTN. Test animals received croton oil 24 hours before the NTG at two dose levels; 2ml/kg and 1ml/kg body weight. The difference between the test and control animals was not signifcant.

There was also no significant difference between the two groups in the 24 hour urine volume passed, the creatinine or urea levels.

III.2.3 Conclusions

Inducing an acute phase response prior to the administration of NTG, did not appear to increase the severity of proteinuria or affect renal function during the heterologous phase, neither when a dose of NTG close to the threshold for proteinuria was used, nor when the injury was more severe.

Since the injection of NTG itself resulted in an acute phase response, although delayed and much less intense (Part II.3.3), it was still not possible to exclude a role for the acute phase response in enhancing injury. As injury in the heterologous phase may be caused by antibody binding alone (although enhanced by the involvement of polymorphonuclear leucocytes), the possible effect of an acute phase stimulus on inflammatory mediators such as macrophages, polymorphonuclear leucocytes and lymphocytes, may be less apparent.

III.3.0 THE EFFECT OF AN ACUTE PHASE STIMULUS ON THE AUTOLOGOUS PHASE OF NEPHROTOXIC NEPHRITIS

The pathogenetic mechanisms involved in producing injury during the autologous phase, more closely approximate human anti-GBM antibody disease than does the heterologous phase. It is more sustained, anti-GBM antibodies are constantly present, damage is more severe, and a

mononuclear cell component appears to be involved. Furthermore, autologous antibody rather than passively administered heterologous antibody is involved.

Unfortunately, the severity of injury and natural course of the autologous phase in rabbits is extremely variable and unpredictable. Giving the same dose of NTG to a homogenous group of rabbits resulted in the production of no overt disease in some, moderate disease in others, and extremely severe diease - sometimes leading to death - in a few. This issue is further analysed in Part V.

The effect of an acute phase stimulus was therefore assessed in three situations during the autologous phase: (a) in rabbits with stable renal function 3 days prior to induction of the stimulus; (b) in rabbits with improving renal function; and (c) in rabbits with deteriorating renal function.

III.3.1 Experimental protocol

Rabbits were housed in metabolic cages and NTN was induced as described in Part II. A dose of 0.5 mg/kg body weight was used for most of the rabbits as this dose is very close to the "threshold" for induction of damage during the autologous phase. It was thought that this was the most sensitive model, as the presence of an acute phase response might precipitate injury in animals in which the NTG dose was just too low to result in consistent injury.

An acute phase response was induced by the injection of croton oil in liquid paraffin as previously described (Part III.1.0) at various times during the autologous phase.

III.3.2 Results

(a) Rabbits with stable renal function (n = 16)

In this group the absolute serum creatine levels on the day that the acute phase stimulus was given ranged from 60 umol/l to 320 umol/l with a mean of 122 umol/l, but was stable for 3 days prior to the induction of the stimulus.

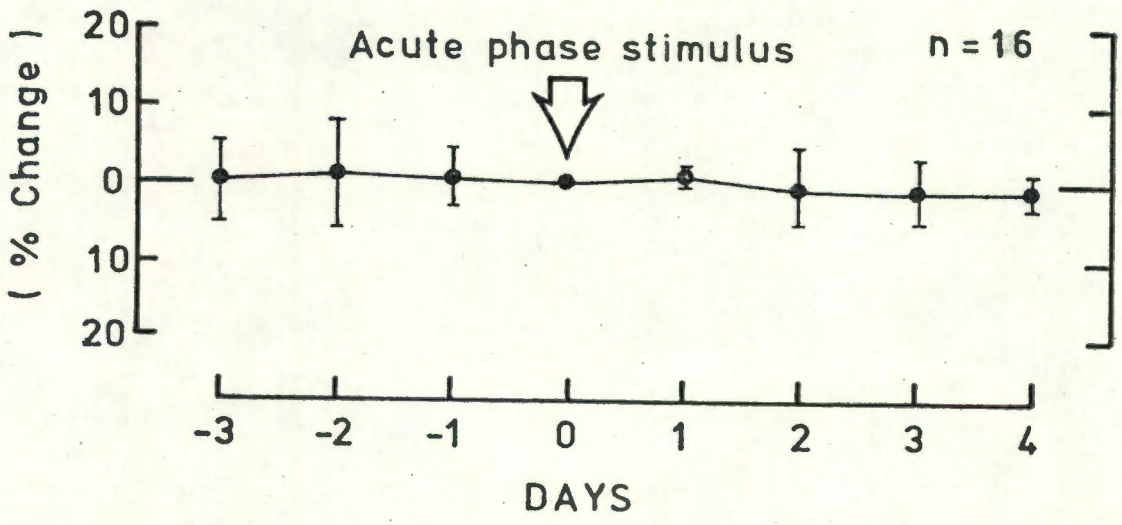


Fig.III.23

Percentage change in serum creatinine following the induction of an acute phase stimulus during the autologous phase. (Mean ± SD)

No significant deterioration of function occurred in any of these rabbits following on the induction of an acute phase stimulus.

(b) Rabbits with improving renal function (n = 9)

In this group the absolute serum creatine levels ranged from 90 umol/l to 250 umol/l with a mean of 149 umol/l.

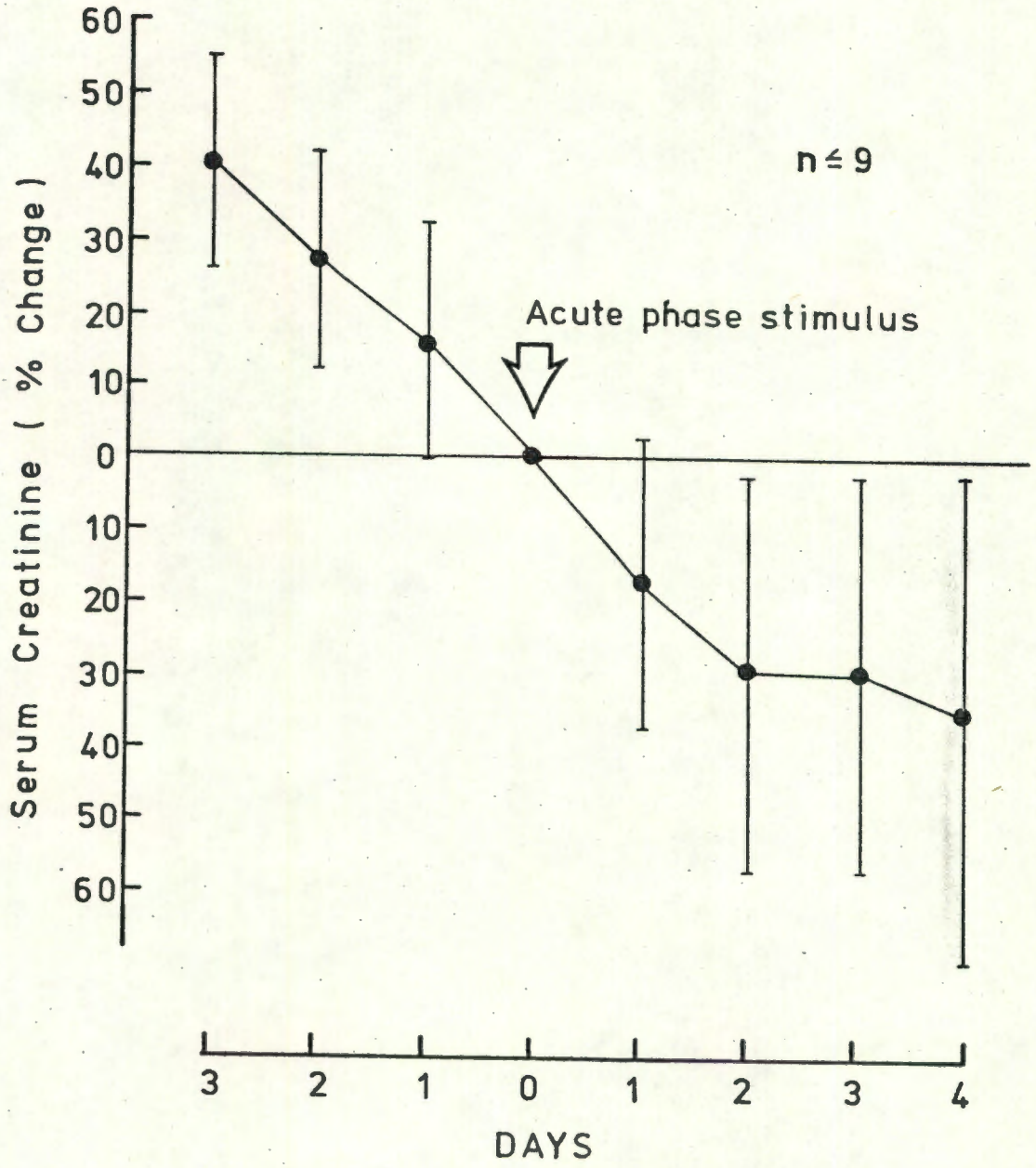


Fig.III.24

The effect of an acute phase stimulus on the course of NTN in rabbits with improving renal function during the autologous phase. (Mean ± SD)

As can be seen from Fig.III.24, function was improving in all rabbits at the time that the stimulus was given and in general continued to improve after the stimulus. There was, however, considerable variation between rabbits.

(c) Rabbits with deteriorating renal function (n = 3)

This group was small and very unstable as the stimulus was given early in the course of the disease at a time when function was deteriorating. In the standard model of NTN, most rabbits after initial deterioration, gradually improve, 4 - 6 days after the onset of this phase.

The absolute serum creatine levels at the time of the acute phase stimulus ranged from 80 umol/l to 450 umol/l with a mean of 226 umol/l.

As can be seen from figure III.24, all rabbits began to improve at about the time that the acute phase stimulus was given, and no deleterious effect of the stimulus was apparent. This group, however, was too small and unstable to permit any firm conclusions to be drawn.

Fig.III.25 /...

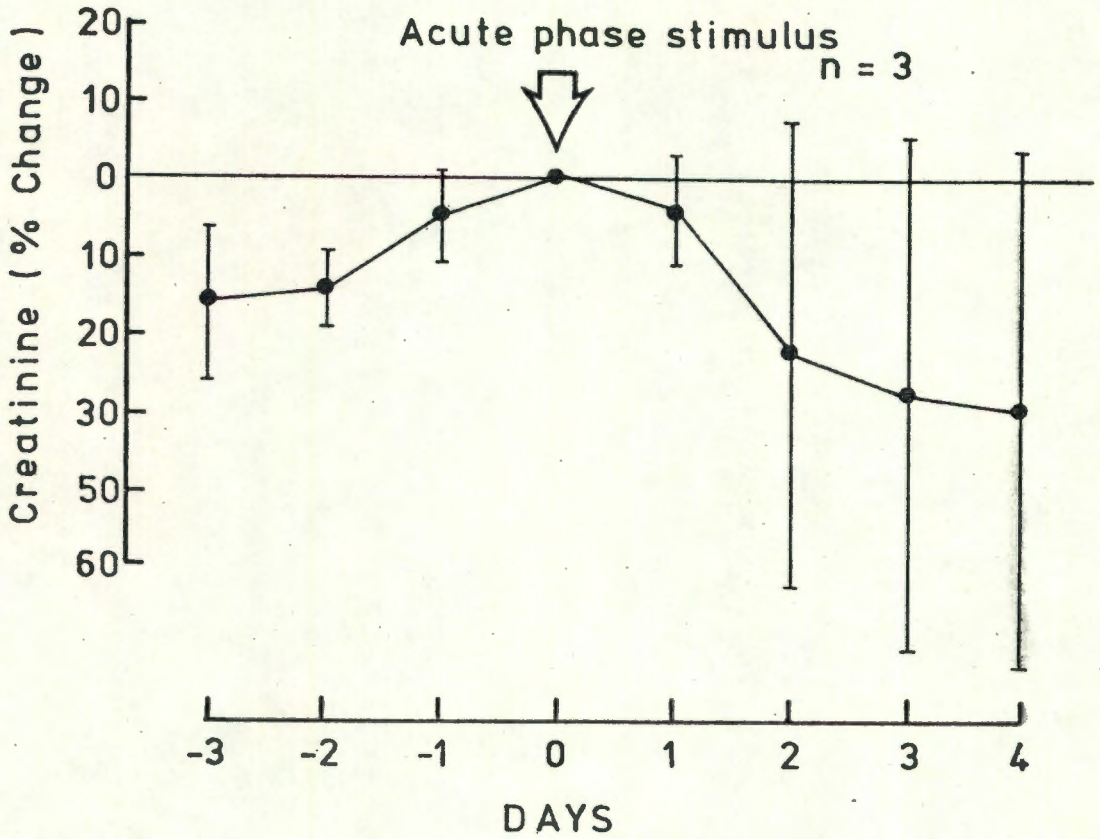


Fig.III.25

The effect of an acute phase stimulus on rabbits with NTN, with deteriorating renal function during the autologous phase. (Mean ± SD)

III.3.3 Conclusions

An acute phase stimulus superimposed at various times on the autologous phase of nephrotoxic nephritis in rabbits with mild, moderate or severe renal impairment did not enhance injury if renal function was stable or improving.

The effect on the disease if given when function was deteriorating, was less certain, even though the limited experimental group also suggested that it had no effect. A very large number of rabbits, possibly with the use of larger doses of NTG and preimmunization of rabbits to ensure a higher proportion with severe disease, would be necessary to resolve this issue. Both test and control groups would be needed and the acute phase stimulus should be given early on in the autologous phase. Lack of adequate quantities of NTG prevented me from doing this experiment. It is likely however that models of NTN with stable or improving function are more representative of the human glomerulonephritides which relapse during infections.

These experiments, however, do not preclude an imporant role for acute phase reactants as inflammatory mediators capable of potentiating disease. They are in any event somewhat raised by the disease (NTN) itself.

From these observations it would appear that the enhancement of injury by intercurrent infections may therefore be due to some factor specifically related to the infection, such as the generation of immune complexes, or a stimulus to inflammatory mediators, different from that produced by a simple acute phase stimulus.

PART IV

STUDIES OF C-REACTIVE PROTEIN DEPOSITION IN GLOMERULI DURING ALLRGIC INJURY

PART IV

STUDIES OF CRP DEPOSITION IN GLOMERULI DURING ALLERGIC INJURY

IV.0.0 INTRODUCTION

C-reactive protein interacts with many of the factors involved in local inflammatory reactions such as complement, platelets and mononuclear cells by enhancing phagocytosis and cell migration (reviewed in Part I.7). Furthermore, it may rise by a factor of up to 3000 at times of local tissue injury, potentially making it a very powerful inflammatory mediator.

In view of its capacity to bind to a wide variety of substances such as phospholipids, cell membranes, polyanions and polycations, the possibility of finding CRP in inflamed tissue was considered, as it could potentially act as a local inflammatory mediator, causing enhanced injury.

IV.1.0 MATERIALS AND METHODS

IV.1.1 Fluoresceine conjugation of anti-rabbit CRP, anti-human CRP and anti-rabbit C3

Anti-rabbit CRP was raised in guinea pigs, using highly purified rabbit CRP obtained from Dr M Pepys. The specifi-

city and purity was assessed by double immunodiffusion as described in Part II.1.18. Anti-human CRP was obtained commercially (Miles Laboratories Inc., U.S.A.) and its specificity also assessed by double immunodiffusion. Goat anti-rabbit C3 was prepared as described in Part II.1.10.

Fluoroisothiocyanate (FITC) monomer I was obtained from BDH Chemicals Ltd., U.K., and dissolved at a concentration of 2 mg/ml in 0.2 M Na_2HPO_4, Na_3PO_4 (pH 9.3) buffer, just prior to conjugation. Fluoresceine labelled rabbit anti-goat IgG was obtained from Miles Biochemicals for use in the indirect immunofluorescent studies (see below).

Method

Conjugation was done according to the method of Wood, Thompson and Goldstein (1965).

Sodium sulphate was added to the antiserum at a concentration of 18% and stirred continuously for 3 hours to allow complete flocculation of the globulin. After centrifugation, the precipitated globulin was washed 3 times in 18% sodium sulphate and finally re-dissolved in a small volume of 0.9% sodium chloride. It was then dialysed for 24 hours at 4°C in cellophane tubing.

The protein concentration was determined by the Folin method (Part I.1.18) and adjusted to 25 mg/ml prior to FITC conjugation.

FITC in phosphate buffer (pH 9.3) was slowly added at a concentration of 1 mg FITC to each 100 mg globulin with 2 ml of buffer (0.2 M Na_2HPO_4, Na_3PO_4 pH 9.3) taking care that the pH did not drop below 9.0, stirring continuously for 1 hour. The pH of the solution was then reduced to 7.6 with 0.2 M NaH_2PO_4.

Fractionation of the FITC-globulin was performed by Sephadex G-25 gel filtration and diethylaminoethyl (DEAE) cellulose anion exchange chromatography.

A 50 ml Sephadex G-25 chromatography column was prepared as described by Fahey and Terry (1973) and equilibrated with 0.01 M phosphate buffer (pH 7.6). The eluted fractions comprising the first protein peak were kept whilst the second protein peak containing unbound FITC was discarded.

A 50 ml DEAE-cellulose chromatography column was equilibrated with 0.01 M phosphate buffer pH 7.6. The FITC-globulin sample was placed on the column and eluted successively with 50 ml of the following three buffers:

(1) 0.01 M phosphate buffer (pH 7.6)

(2) 0.01 M phosphate buffer (pH 7.6) containing 0.2% NaCl

(3) 0.01 M phosphate buffer (pH 7.6) containing 0.9% NaCl

Only the fraction from the protein peak of the third buffer elution, containing high concentrations of purified FITC-labelled immunoglobulin were kept.

These preparations were then adsorbed with pig liver powder (Wellcome Research Laboratories, U.K.) using 1/5 powder to

volume, of FITC-globulin. Anti-rabbit CRP was further adsorbed by the addition of 1 : 2 normal rabbit serum, free of CRP.

The FITC labelled anti-C3 serum was tested for specificity and potency of staining by direct immunofluorescent microscopy of kidneys known to contain C3. Non-specific tissue staining was prevented by dilution of anti-serum prior to use. The FITC anti-rabbit CRP and anti-human CRP was tested for specificity by double immunodiffusion in gels (Ouchterlony 1958) and by staining of CRP-coated sepharose beads. Abolition of staining was achieved following adsorption of antiserum with a slight excess of a CRP solution. Negative reactions were obtained with all normal kidney sections studied.

IV.1.2 Fixation and staining techniques

Because of the marked solubility of CRP, usual techniques of fixation employing alcohol, ether or acetone causes extraction of CRP and consequently poor staining, possibly also due in part to denaturation of the CRP by the conditions of fixation (Kushner 1961).

The following procedure was therefore employed (as recommended by Kushner). Fixation was accomplished by the use of a 1.0 % picric acid in acetone mixture, neutralized by saturation with sodium sulphate crystals. Freshly cut air-

dried sections were immersed in this solution and gently shaken at room temperature for 1 hour. The slides were then washed with continuous shaking in three changes of anhydrous acetone for 1 1/2 hours to remove excess picrate and air-dried at 37°C for 30 min. The sections were then overlaid with 1 to 2 drops of fluoresceine conjugate for 1 hour in a moist chamber, washed for 10 minutes in two changes of buffered saline (0.01 M phosphate pH 7.2) and mounted in glycerol buffer.

Fluorescent microscopy was performed as described in Part II.1.20.

IV.2.0 EXPERIMENTAL PROTOCOL

Fresh renal biopsies were obtained from as many sources as possible for this study.

IV.2.1 Studies on renal biopsy samples of rabbits with experimental nephritis

(a) Heterologous phase of NTN (3 rabbits)

Kidneys were removed 48 hours after the injection of 2 ml/kg of NTG. All rabbits had heavy proteinuria at the time of sacrifice.

(b) Autologous phase of NTN (2 rabbits in each group)

Groups (i) Kidneys removed on day 5

(ii) Kidneys removed on day 12

(iii) Kidneys removed on day 28

Animals in groups (ii) and (iii) all had severe disease at the time.

(c) Experimental immune complex disease (2 rabbits)

Kidneys were obtained from Dr. Bruce Pussel, working in the same laboratory on rabbits given large doses of aggregated human IgG, which resulted in particularly severe injury to the kidneys.

IV.2.2 RESULTS

In none of the kidneys examined was CRP detected. Only in the kidneys taken from rabbits with experimental immune complex disease was mild CRP positive staining noted in the lumen of some blood vessels, possibly reflecting CRP contained in thrombi in the vessels.

All sections examined had heavy staining for C3 and rabbit IgG (anti-sheep), where these were anticipated. These features are illustrated in Table IV.8.

Stain	Site	Heterologous phase	Autologous phase Day			Experimental immune complex disease
		48 hrs	5	12	28	
C3	glomerular	+++	+++	+++	+++	+++ (granular)
	tubular	±	±	±	±	-
Rab IgG	glomerular	-	+	+++	++	
	tubular	-	-	-	-	
CRP	glomerular	-	-	-	-	+ in vessel
	tubular	-	-	-	-	lumina
Indirect IF for CRP	glomerular	-	-	-	-	-
	tubular	-	-	-	-	-

Table IV.8

Direct and indirect (FITC) labelled antibody staining for CRP, C3 and rabbit IgG in kidneys taken during various stages of nephrotoxic nephritis and in immune complex nephritis. All staining was linear except in the case of the immune complex disease where heavy granular deposits were noted in capillary loops and in the mesangium.

In view of the negative results, stains on the same sections were also done, using an indirect immunofluorescent technique as described in Part II.1.20. Fluoresceine labelled rabbit anti-goat antiserum was used to detect the goat anti-rabbit CRP which was used as the direct antibody applied to the kidney slices. Uniformly negative results were again obtained (Table IV.8).

IV.2.3 Studies on human renal biopsy samples

Small samples of biopsies taken from humans for diagnostic purposes were also obtained and processed by the techniques as described above (Part IV.1.2).

Patients with the following diseases were included in this study:

(1) Wegener's granulomatosis
(2) Mesangial IgA disease x 2
(3) Transplant rejection
(4) Systemic lupus erythematosus x 2
(5) Henoch-Schonlein purpura

CRP again could not be demonstrated in any of these samples, by using the techniques of direct and indirect immunofluorescent microscopy.

These findings were therefore consistent with those of the animals studies. It is recognized however, that the range of human material studied was limited, due to lack of suitable sections.

IV.2.4 Discussion

Kushner (1961) was first to describe the finding of CRP in the inflammatory reactions induced by typhoid vaccination and subsequently also in experimental myocardial infarction (1966). Parish (1971, 1976) also described its presence in the lesions of certain types of acute and chronic

vasculitis. However, it was found more often in lesions infiltrated by neutrophils such as necrotizing vasculitis (19 out of 26 cases) than in those with predominantly mononuclear cell changes (17 out of 32 cases). Most of the CRP found was also noted intravascularly or involving the endothelium.

C-reactive protein therefore does not appear to be a universal finding in inflamed tissue. To the best of my knowledge, no one has been able to demonstrate its localization in glomeruli, except possibly in some varieties of SLE nephritis (De Beer, F.C., personal communication).

These findings do not exclude a local role for CRP in inflammatory lesions. Its main function however, is probably the binding to damaged cell membranes and tissue components, allowing for their effective opsonization and removal by the monocyte phagocytic system.

PART V

RATE OF ANTIBODY DEPOSITION AS A POSSIBLE DETERMINANT OF INJURY

PART V

RATE OF ANTIBODY DEPOSITION AS A POSSIBLE DETERMINANT OF INJURY

V.0.0 INTRODUCTION

From observations made during many episodes of autologous phase nephritis, it became clear that in groups of rabbits all receiving the same dose of NTG, some developed severe nephritis whilst others remained well.

The following experiments were done to investigate the paradox of high serum concentrations of autologous antibody directed towards the heterologous anti-glomerular basement membrane immunoglobulin, without the development of overt injury.

The findings emphasize the importance of the rate of antibody deposition in causing injury and show how initial low titres of antibody appear to "protect" against the effects of subsequent high titres of antibody. These data also illustrate certain aspects of the effector function of immunoglobulins.

V.1.0 MATERIALS AND METHODS

The standard protocol for the induction of nephrotoxic nephritis, collection and storage of samples was used as described in Part II.

V.1.1 Measurement of the rate of elimination of NTG from the circulation

Daily, 1 ml samples of plasma were precipitated with an equal volume of 20% trichloracetic acid (TCA) and the radio-activity from the ^{125}I isotope counted in a Packard gamma counter. This level of protein bound isotope was used as a measure of the sheep immunoglobulin remaining in the circulation.

V.1.2 In vitro assay of the ability of the NTG bound to the GBM to react with rabbit anti-sheep IgG

Normal sheep IgG was prepared by adding sodium sulphate to sheep serum at a concentration of 18%. This mixture was stirred continuously for 3 hours to allow complete flocculation of the globulin. The precipitate was washed 3 times in 18% sodium sulphate, re-suspended in 0.9% sodium chloride and dialysed against 0.01 M phosphate buffer pH 7.6 overnight. It was further purified by passage down a DE52 column equilibrated with a 0.01 M phosphate buffer pH 7.6. This purified IgG was homogenized with Freund's complete adjuvant (FCA) and injected into rabbits; this was repeated after two weeks using incomplete adjuvant (FA).

The animals were bled out and the rabbit anti-sheep IgG antibody assessed using the Ouchterlony technique (Part II.1.18).

This antibody was then purified by sodium sulphate fractionation and passage down a DE52 column, after which it was labelled with ^{125}I using the chloramine-T method described in Part II.1.7.

Normal rabbit IgG was purified in a similar way and labelled with ^{131}I to act as a plasma marker in this sytem.

Kidneys were obtained from rabbits killed on day 2 and 20 after IV NTG, whilst normal rabbit kidneys were used as controls. The renal cortex was separated from the medulla and the cortex was then ground to a fine sludge in a glass homogenizer and washed three times in PBS, centrifuging between the washes at 5000 r.p.m. for 15 min.

Test: Normal rabbit kidney and kidneys taken 2 and 20 days after injection of the NTG were pre-incubated with normal rabbit IgG at 37°C for 30 minutes to reduce non-specific binding of the radio-labelled antibody to the homogenates. These samples were then incubated with the ^{125}I labelled rabbit anti-sheep antibody for 30 minutes, in combination with the ^{131}I labelled normal rabbit IgG.

Three amounts of this labelled antibody at a concentration of 25 mg/ml were used: 5 ul, 50 ul and 100 ul, which were added to the following volumes of kidney homogenates:

100 ul	Day 2 (heterologous phase kidneys)
220 ul	Day 20 (autologous phase kidneys)
220 ul	Normal kidneys (controls)

These volumes were used in order to keep the total amount of membrane bound sheep IgG constant, less being present on day 20 compared to day 2. - These quantities had been determined by earlier experiments.

^{131}I labelled normal rabbit IgG was used in each case as a non-specific marker. The tubes were incubated at 37°C for 30 minutes, the sediment washed 4 times in PBS and the radioactivity of the isotopes counted.

The amount of fixed antibody was calculated using the following formula:

$$\text{ug antibody bound} = \frac{X - \left[\frac{L}{M}\right] \times Z}{Y}$$

where X = ^{125}I kidney counts

Y = ^{125}I counts per ug of NTG injected

L = ^{125}I counts per ml of serum

M = ^{131}I counts per ml of serum

z = ^{131}I kidney counts

Measurements of urine protein, urea, creatine, C3 were done as described in Part II.1.18.

Light and electron microscopy were done as described in Part II.1.20.

V.2.0 EXPERIMENTAL PROTOCOL - AUTOLOGOUS PHASE

Animals were housed in metabolic cages and their urine was collected continuously. After two days' observation to establish baseline values for protein excretion, animals were injected with NTG labelled with ^{125}I together with ^{131}I normal sheep globulin as a plasma marker.

From previous experiments it was possible to choose a dose of NTG just sufficient to result in autologous nephritis in some rabbits and not in others. This dose was 0.5 ml/kg body weight which caused the binding of approximately 75 ug of antibody per kidney. This amount of antibody binding was below the level needed to produce proteinuria during the heterologous phase and was expected to cause autologous phase disease in about 50% of the rabbits.

Daily samples of blood and urine were collected and stored as described in Part II.1.2.

Twenty-one days after injection, the animals were killed and allocated to one of two groups depending on the severity of the resulting disease.

Group A had definite autologous phase injury which was defined as:

(i) Protein excretion of more than 200 mg per 24 hours or

(ii) Doubling of either plasma urea or creatinine levels.

Group B had no injury as defined by these criteria.

V.2.1 RESULTS - AUTOLOGOUS PHASE

There were no differences in plasma urea, creatinine, C3 or protein excretion before injection of nephrotoxic globulin and none of the animals developed proteinuria during the heterologous phase.

During the autologous phase animals were easily separated into Group A (15 animals); and Group B (7 animals) The results of this grouping are shown in figure V.26 and figure V.27.

Fig.V.26 & 27 /...

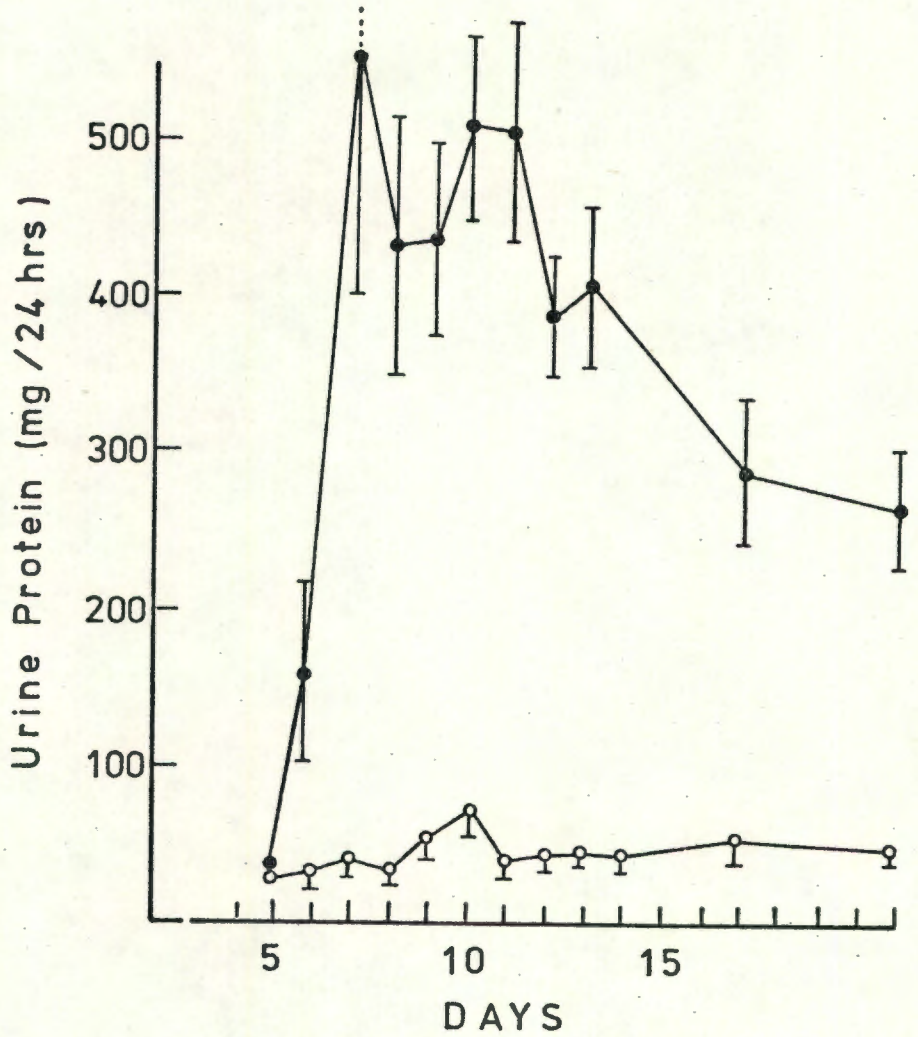


Fig.V.26

Proteinuria during the autologous phase. (Mean ± SE) Rabbits were divided into Group A (n=15), with severe disease (●), and Group B (n=7), with minimal disease (o). All rabbits had received the same dose of NTG at the onset of the experiment (0.5 ml/kg body weight). These differences were highly significant ($p < 0.001$).

Fig.V.27/...

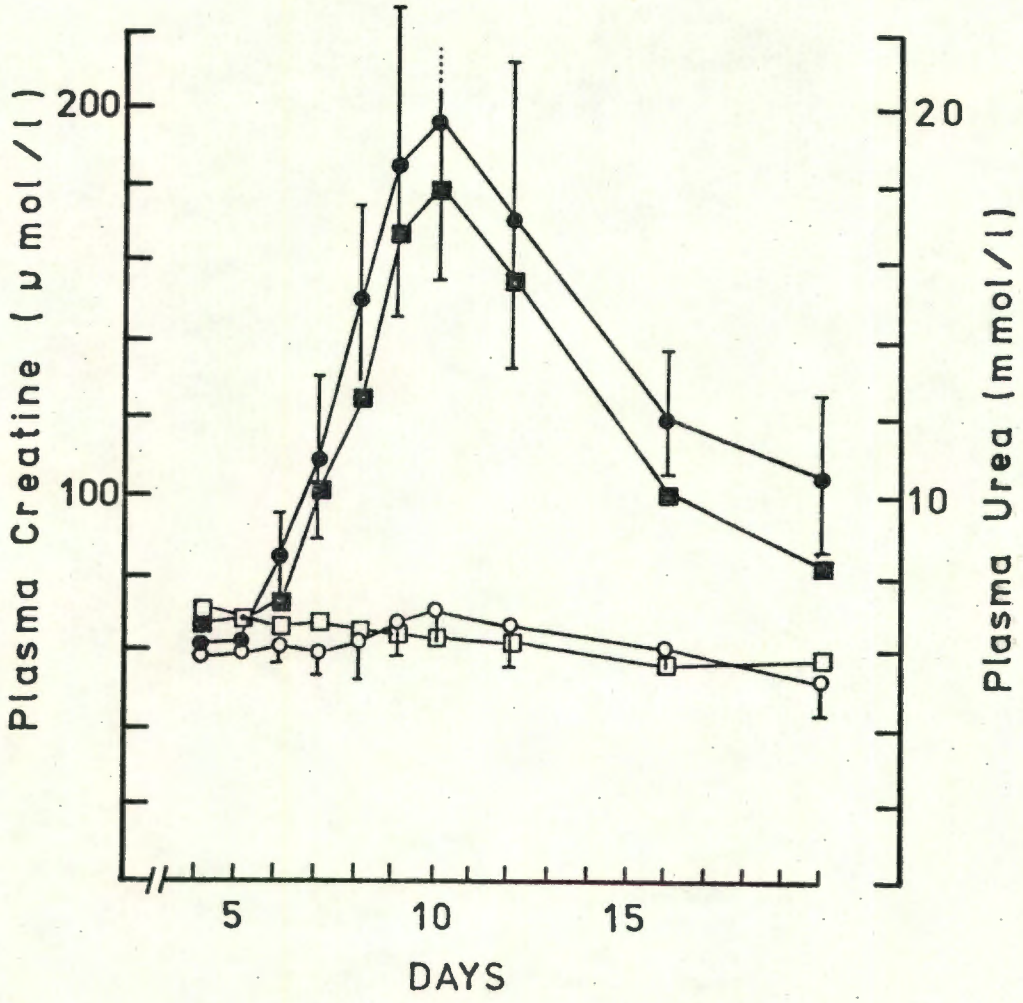


Fig.V.27.
Renal function during the autologous phase. (Mean ± SE)
Serum creatinine (■) and serum urea (●), in Group A animals compared to those of Group B. Creatinine (□) and urea (o). These differences were highly significant ($p < 0.001$).

V.2.2 Autologous anti-sheep antibody response

All the rabbits developed antibodies to sheep IgG as can be seen in figure V.28.

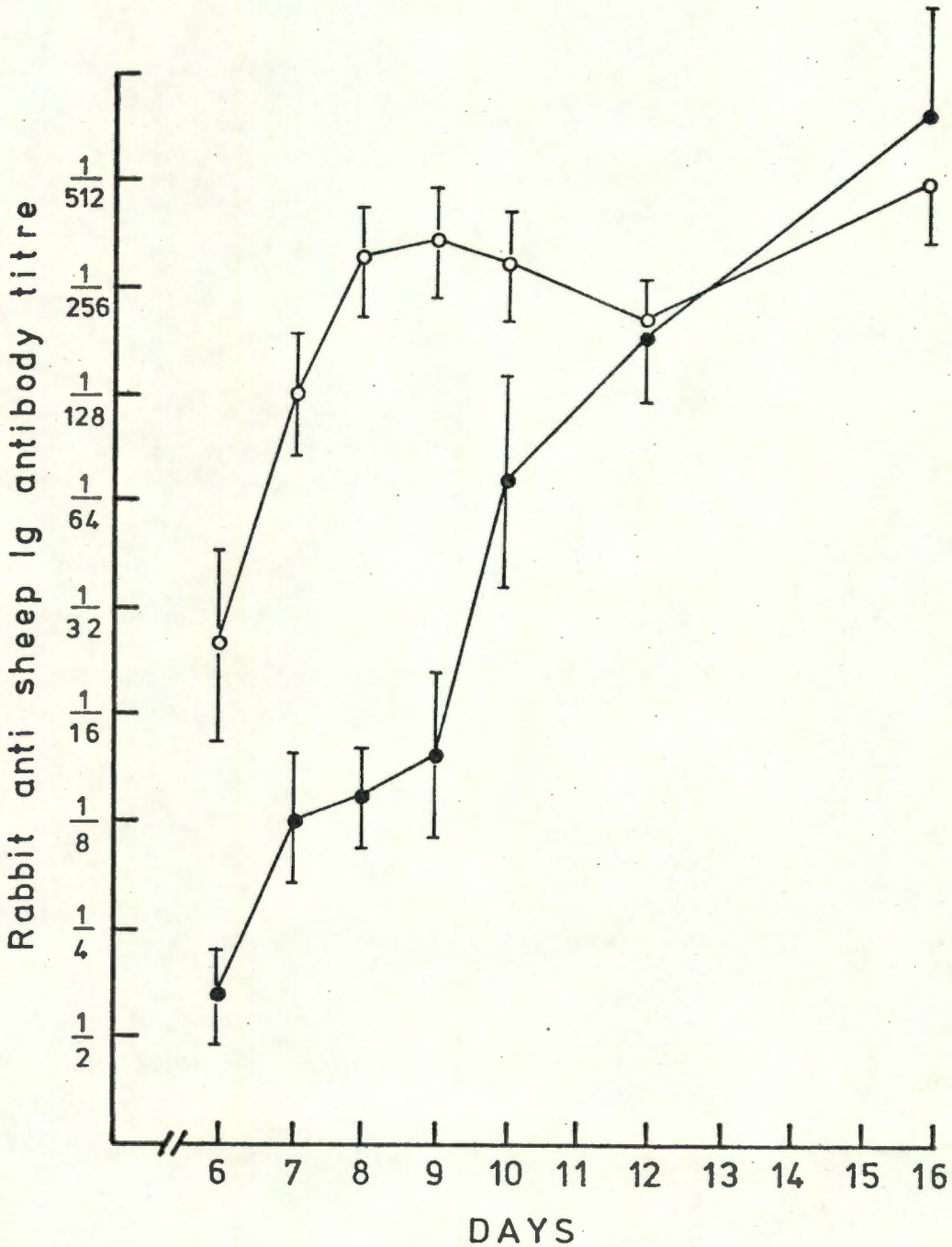


Fig.V.28

Autologous antibody response to sheep IgG, mean $^{+}$ SE. There is a significant difference in antibody titre between Group A (○), and Group B (●), on day 8 ($p < 0.02$) Wilcoxon Rank Sum Test.

The titres of autologous rabbit anti-sheep antibodies in animals with definite autologous phase injury (group A) rose more rapidly and were higher on each day from the onset of detectable injury (day 6) until day 10 when protein excretion was greatest. The difference in antibody titres on day 8 was significant $p < 0.02$.

After day 10 there was no difference in autologous antibody titres between the groups. The late rise in titre (days 9 to 11) in group B animals was not associated with overt injury.

The more rapid rise of antibody in Group A is emphasized by integration of the anti-sheep antibody titre curve over the 48 hours after their appearance. This type of analysis is illustrated in figure V.29. Group A had a mean value of 93.6 , (SD = 42) compared to 48.5 , (SD = 18) for group B. This difference is statistically significant ($p < 0.01$ Wilcoxon Rank Sum Test).

Fig.V.29/...

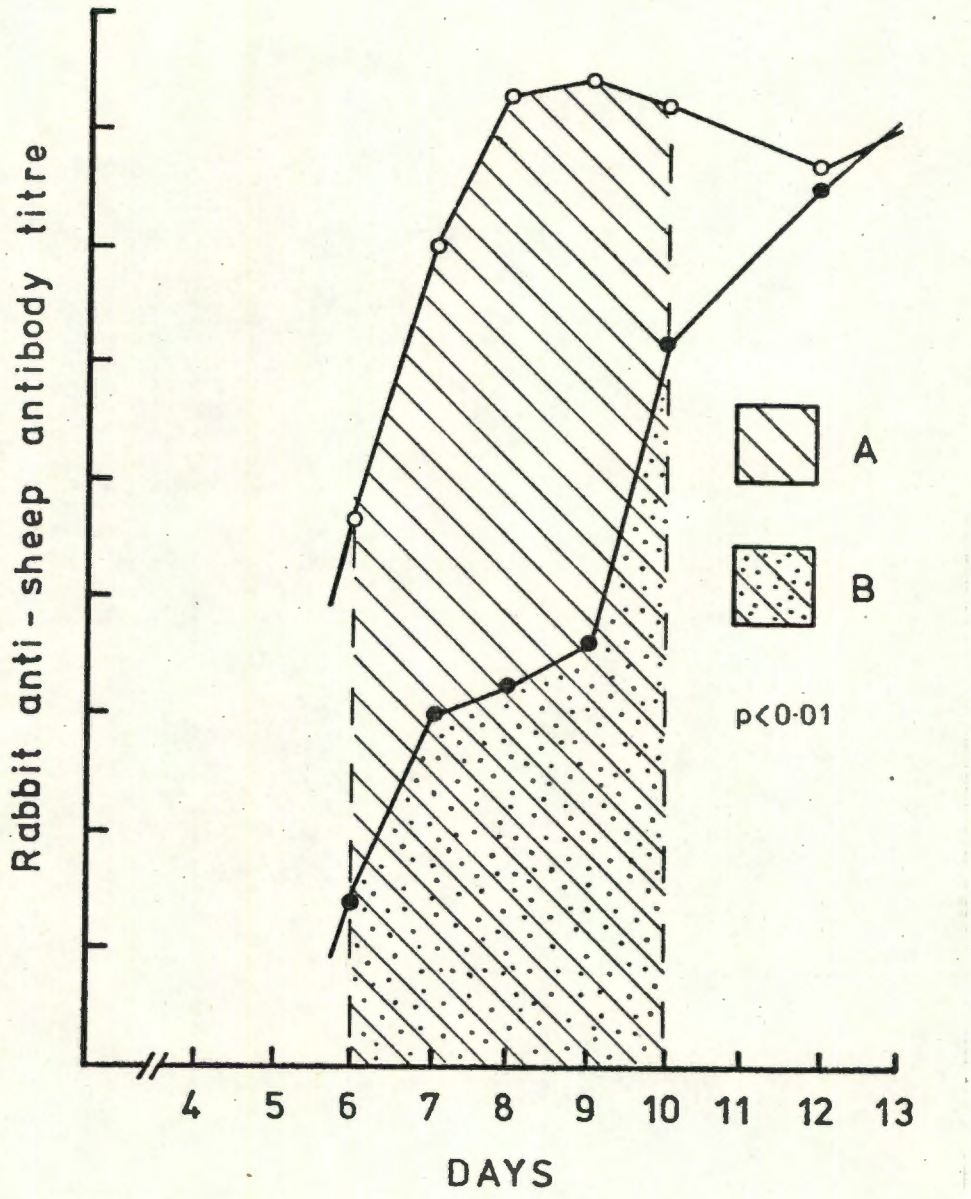


Fig.V.29

Integration of the area under the antibody titre curve in animals of Group A (▨), and Group B (▨).

As can be seen there was a striking difference between those animals which developed disease and those which did not.

V.2.3 Disappearance of sheep globulin from the circulation

Another index of reactivity to sheep globulin was derived by the daily measurement of the TCA precipitable ^{125}I labelled NTG remaining in the plasma, i.e. the sheep nephrotoxic globulin. During the first 4 days disappearance was exponential with a half life (T1/2) of 23.2 days, due to normal catabolism of the protein. The onset of "immune elimination" was noted when the rate of disappearance increased, as illustrated in figure V.30.

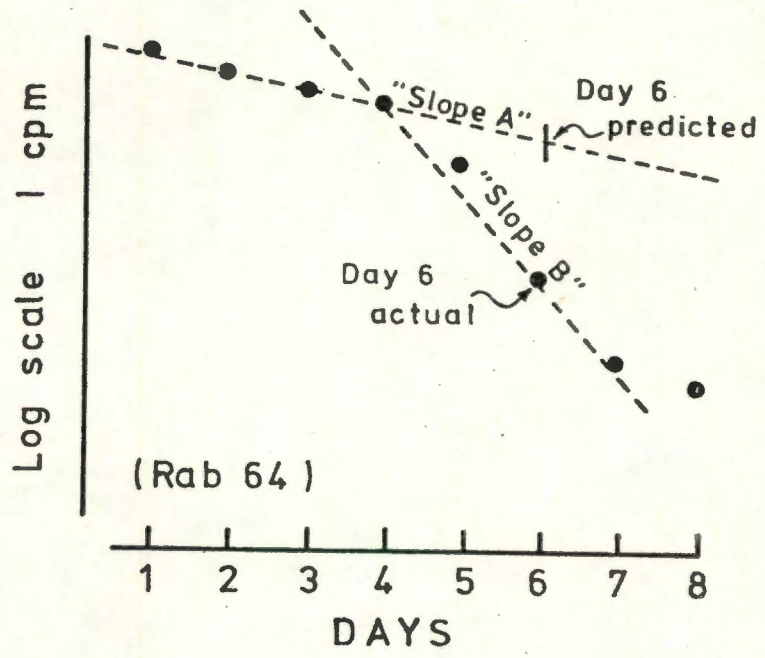


Fig.V.28

Example of the technique used to demonstrate the rate of disappearance of NTG from the circulation due to normal catabolism ("slope A"), compared to that produced by "immune elimination" ("slope B"). Rabbit no.64 used as an example.

Immune elimination was more rapid in rabbits from group A This difference was most noticeable on day 7, when the difference between actual and predicted log c.p.m. ^{125}I was significantly greater in group A animals (1.31 SD = 0.73) than in group B (0.38 SD = 0.73) ($p < 0.01$, Wilcoxon Rank Sum Test) (Fig.V.31).

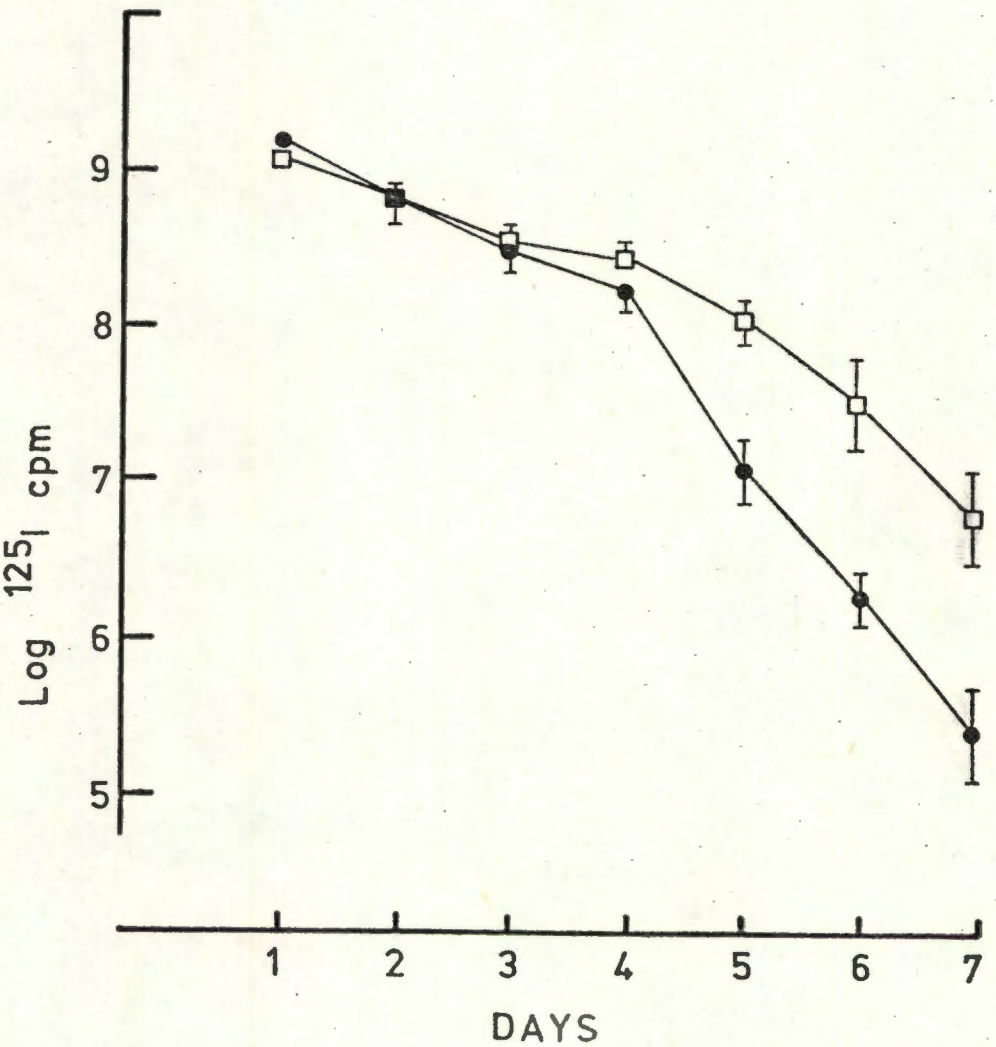


Fig.V.31

Disapperance of ^{125}I labelled NTG from the circulation in Group A animals (●), compared to that of animals in group B (□). The difference is significant ($p < 0.01$).

V.2.4 Other observations

(a) The C3 level fell to a greater extent in group A animals (74 % + 2 %) than in group B (81 % + 17 %) this difference was however not statistically significant (Fig.V.32).

(b) Residual NTAb fixed in the kidney at 21 days was less in Group A animals, 50 ug (SD = 9.9) compared to 65 ug (SD = 15) in group B, again the difference was not statistically significant (Fig.V.32).

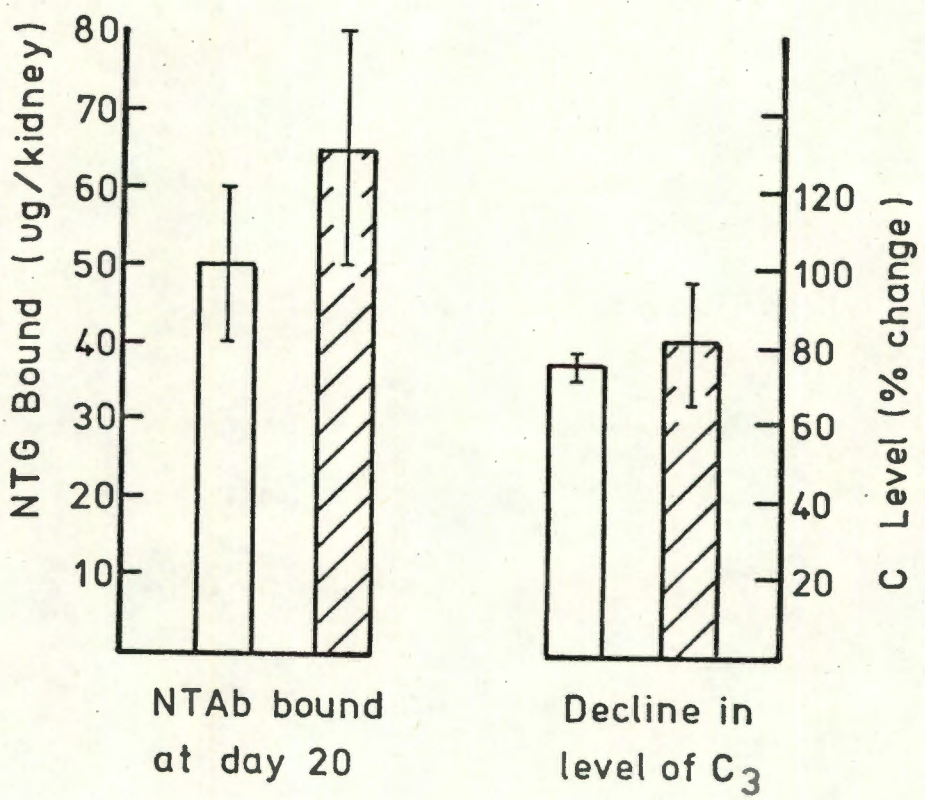


Fig.V.32

Comparison between the amount of NTAb bound on day 20 and the maximum drop in C3 levels noted during the autologous phase in Group A (▭) and Group B (▨) rabbits. Mean ± SD. The differences were not significantly different.

(c) Quantitative histology was performed on kidneys from 4 randomly selected rabbits from each group.

There was no difference in glomerular PMN count but there was less proliferation and no crescents in animals from group B, as can be seen from table V.8.

	Polymorphs			Hyper-cellularity			Crescents			
	+	+ +	+ + +	+	+ +	+ + +	0	+	+ +	+ + +
A		2	2		1	3	1	1		2
B		1	3	1	2	1	4			

Table V.8

Histology on kidneys removed on day 20 from rabbits in groups A (n = 4) and B (n = 4)

(d) Immunofluorescent studies showed no difference in the pattern or intensity of staining of both groups, all animals had deposition of sheep IgG, rabbit IgG and C3.

V.2.5 Conclusions

These results suggest that the initial rate of rise of rabbit anti-sheep antibody and the attainment of early high titres determine the extent of autologous phase injury and that subsequent rises in antibody titres have little effect on disease activity.

These findings suggest an important role for the rate of antibody deposition as a determinant of injury and also possibly indicate a "protective role" for the early low titre antibodies in saturating the available antigenic sites of NTAb fixed to the GBM, preventing the subsequent fixation of sufficient antibody from reaching a threshold level capable of initiating further damage.

Further experiments were done to test both propositions more directly.

V.2.6 In vitro studies

It would be expected that kidneys removed early during the heterologous phase would have GBM bound sheep IgG without any attached rabbit IgG, as was clearly demonstrated in Part II.3.8, whilst kidneys removed late in the autologous phase would have considerable quantities of autologous Ab bound to the sheep NTAb.

In order to determine whether the sheep nephrotoxic Ab bound to the rabbit GBM could become "saturated" and therefore no longer react with autologous anti-sheep Ab, the following experiments were done. The in vitro binding of rabbit anti-sheep antibodies to homogenates of normal kidney and kidneys removed on days 2 and 21 following IV NTG, was measured as described in Part V.1.2.

RESULTS

The specific binding of rabbit anti-sheep IgG to homogenates of normal rabbit kidney was minimal at all 3 doses used. In contrast, the binding to kidneys removed 2 days after injection of NTG was increased above background approximately two-fold with 5 ul of antibody; five-fold with 50 ul; and twenty-fold with 100 ul. Kidneys removed 21 days after NTG showed no such increase of specific binding. The differences in binding were highly significant ($p < 0.001$) (Fig.V.33).

This confirmed the postulate that the available binding sites on the NTAb, a "planted antigen", present in limited quantities, becomes saturated during the autologous phase limiting the quantity of autologous Ab which may fix, late in the disease.

Fig.V.33 /...

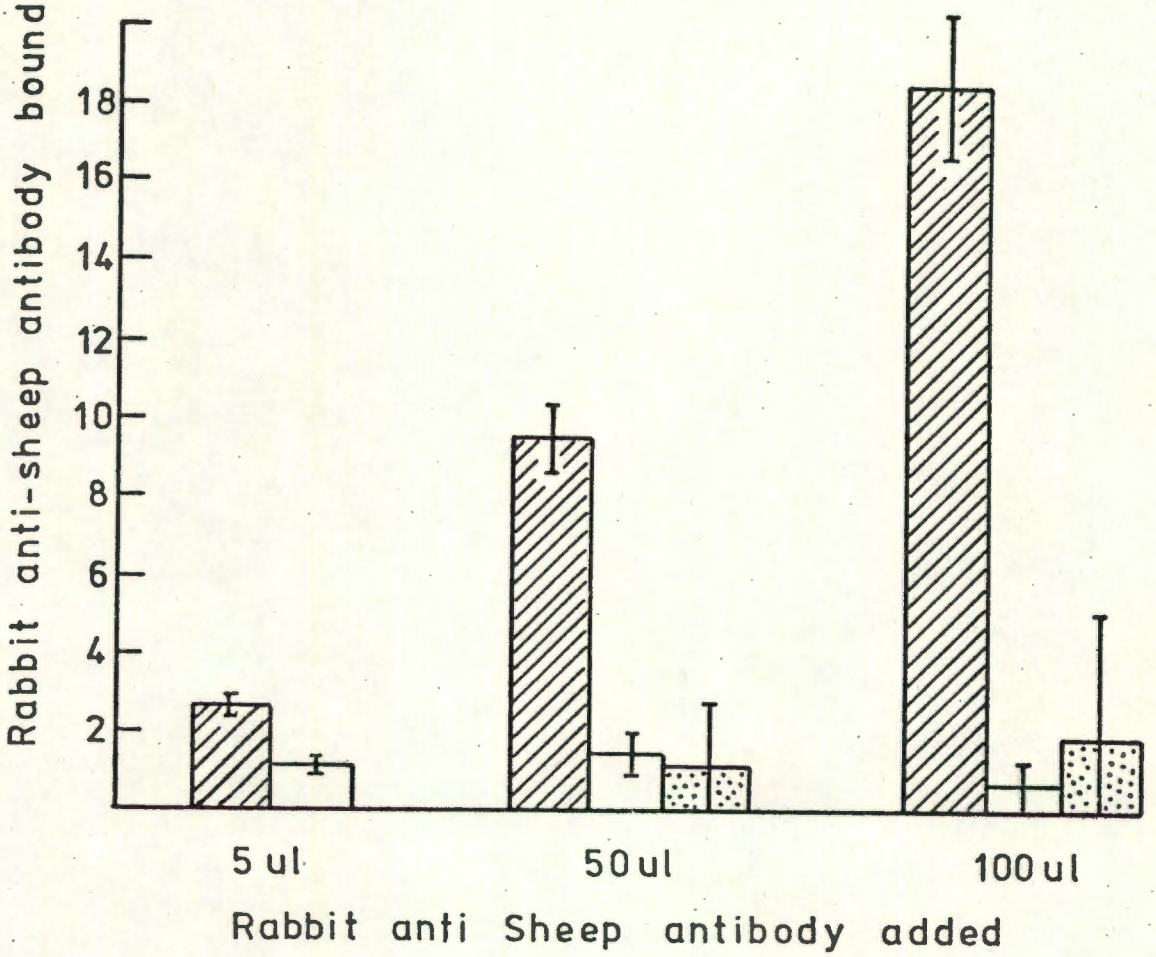


Fig.V.33

Binding of rabbit anti-sheep antibody to homogenates of kidneys removed early during the heterologous phase (////), on day 20 of the autologous phase (▒▒▒) and to normal rabbit kidney (——) Mean ± SD. The differences are highly significant (p , 0.001)

The importance of the rate of fixation of antibody was further investigated with heterologous phase experiments.

V.3.0 HETEROLOGOUS PHASE EXPERIMENTS

V.3.1 Introduction

For these experiments a dose of NTG just sufficient to produce proteinuria was chosen. If, therefore, antibody effector function decayed rapidly, a dose of NTG given slowly might not produce a sufficient density of functional Fc receptors to cause injury.

V.3.2 Results

Two groups of 5 rabbits each were sedated at the start of the experiment with Ketamine HCl ("Ketalar", Parke Davis Laboratories) 1 ml i.m.i. and thereafter with Pentobarbitone ("Sagatal" MayBaker) 0.5 ml IV whenever needed. This sedation was maintained for 6 hours whilst the IV infusion was in progress, thereafter animals were returned to their cages for the next 24 hours after which they were killed, blood samples taken and both the kidneys removed for analysis.

Rabbits were catheterized 6 hours after the NTG infusion was begun to ensure an empty bladder at the start of the 24 hour urine collection period. From then on all urine passed was collected in metabolic cages (specially modified to ensure virtually perfect collections of urine) and 24

hours later rabbits were again catheterized to ensure complete collection of urine.

The nephrotoxic globulin (NTG) used was labelled with ^{125}I, the activity being adjusted so that each rabbit would receive a total dose of about 5 uCi ^{125}I. The normal sheep globulin was labelled with ^{131}I with activity adjusted to give each rabbit about 2 uCi of ^{131}I (Part II.1.7).

Group 1 (control group) (n = 5)
These rabbits each received 1 ml/kg body weight NTG as an IV bolus infusion over approximately 60 seconds. Thereafter they received a total of 1 ml/kg body weight of normal sheep globulin by slow IV infusion over the next 6 hours and were then returned to their cages for 24 hours.

Group 2 (test group) (n = 5)
These rabbits each received 1 ml/kg body weight of normal sheep globulin as an IV bolus infusion over approximately 60 seconds. Thereafter they received a total of 1 ml/kg body weight of NTG by slow IV infusion over the next 6 hours in an identical way to the infusion of normal globulin in group 1.

The IV lines were kept open throughout the 6 hours by slow IV infusion of plasmalyte B.

The kidneys removed at the end of the experiment were homogenized separately, washed, and the amount of NTG which bound was determined as described in Part II.1.19.

Results

Rabbits receiving a fast infusion of nephrotoxic globulin had more proteinuria during the ensuing 24 hours than rabbits receiving an equivalent dose of NTG by slow infusion (Fig.V.35). Rabbits were paired for this analysis according to the amount of nephrotoxic antibody bound, as this was shown to correlate very closely to proteinuria produced during the heterologous phase. (Part II.3.1). This phenomenon was noted despite the fact that the animals receiving the slow infusion in general had more bound antibody yet less proteinuria Table V.9.

Fig.V.35 /...

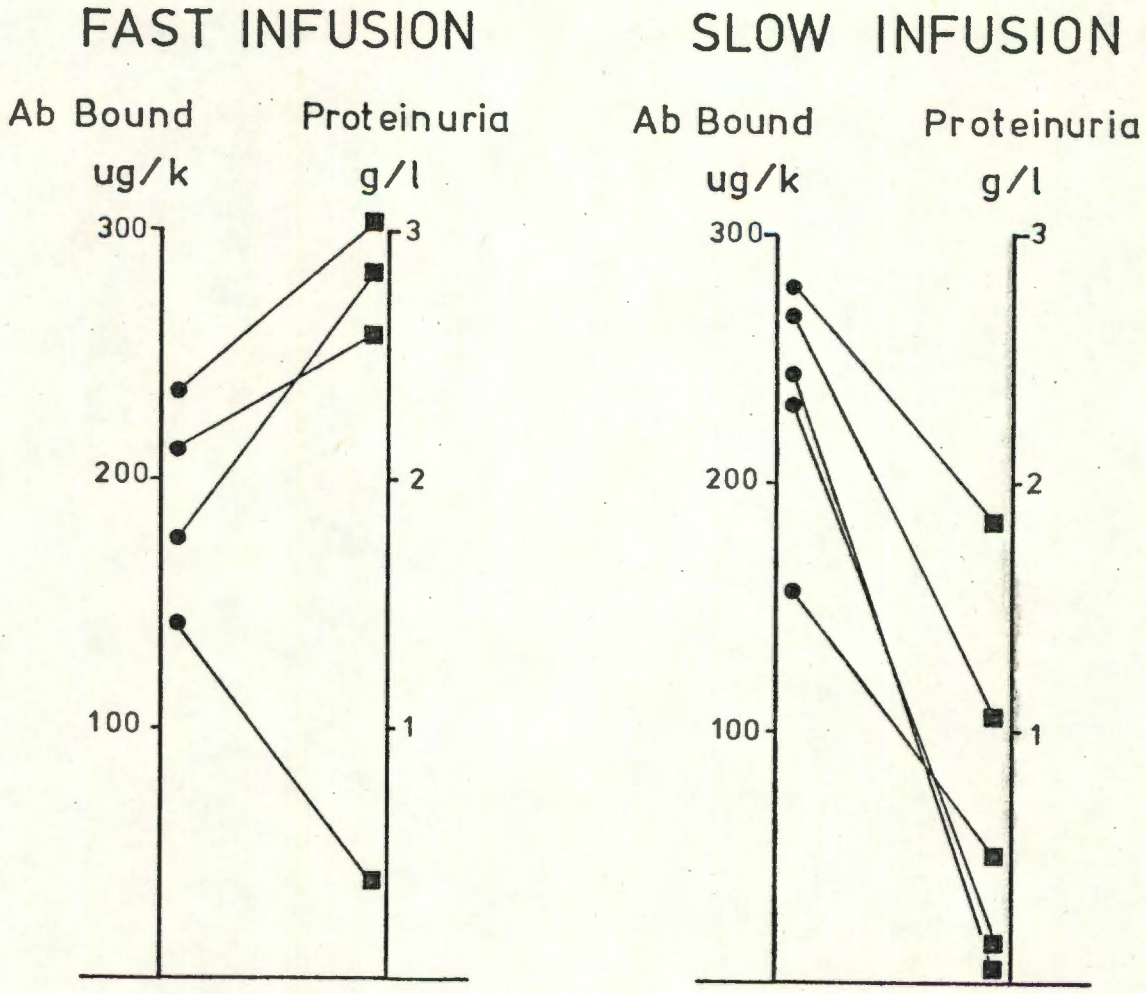


Fig.V.35

Heterologous phase proreinuria produced by the rapid infusion of NTG, compared to that of a slow infusion over 6 hours. NTAb bound, ug/g renal cortex, expressed as the mean of the left and right kidneys.

Table V.9 /...

Rabbit no.	Control Fast infusion					Rabbit no.	Test Slow infusion				
		Antibody bound						Antibody bound			
		µg	x̄	µg/gm k	x̄			µg	x̄	µg/gm k	x̄
11	K1	232	236	34.6	32.9	12	K1	235	232	39.1	39.5
	K2	240		31.8			K2	229		39.8	
14	K1	215	212	30.8	30.8	13	K1	249	246	41.8	41.3
	K2	210		30.9			K2	243		40.8	
16	K1	154	148	22.0	21.0	15	K1	265	278	43.4	43.7
	K2	143		20.1			K2	292		44.0	
17	K1	173	176	15.0	14.5	18	K1	157	154	20.0	19.8
	K2	179		14.1			K2	152		19.6	
20	Died			-	-	19	K1	223	247	23.0	23.5
							K2	271		24.0	
Mean					24.6						33.5

Table V.9

The effect on antibody binding of NTG administered slowly, over 6 hours - to the Test group, compared to a fast infusion, given over 5 minutes to the control group. Mean antibody binding (x). Antibody / kidney (ug x). Antibody / g kidney (ug/g k x).

V.3.3 Discussion

Unanue and Dixon (1967) first described the phenomenon whereby occasional, apparently normal, rabbits fail to develop autologous phase injury after the injection of appropriate doses of NTG. The reasons for this could be clinically important and account for the finding that

certain patients with antibody mediated nephritis have persistently elevated anti-GBM antibody titres without active nephritis.

In the autologous phase experiments documented above, a small dose of NTG which fixed about 75 ugm NTAb to the kidney was used. This resulted in an unusually high proportion (7/22) of animals which failed to develop a definite autologous phase and provided the conditions necessary to study this type of injury.

The most important determinant of autologous phase injury appeared to be the initial rate of rise of autologous rabbit anti-sheep antibody titre and by inference, the initial rate of binding of this antibody to sheep IgG fixed to the GBM. Subsequent antibody titres would then have little effect on the development of autologous phase injury. In rabbits with NTN, autologous phase injury is characterized by infiltration of the kidney with polymorphs (PMN) and monocytes which are engaged by the Fc portion of antibody molecules (Holdsworth et al 1981). In these experiments it seems possible that autologous antibody bound more slowly to the GBM in animals without overt autologous phase injury; and that this provided an insufficient stimulus for engaging polymorphs and monocytes.

The conclusion that the rate of antibody deposition is

important, is supported by the results of the experiments on the heterologous phase of NTN in which proteinuria is caused by Fc dependent infiltration of the glomerulus by PMNs (Cochrane 1965, discussed in Part I.3). In these experiments binding of NTAb after bolus injection - probably complete after 10 to 20 minutes (Unanue 1965) - provoked significantly more proteinuria than similar amounts of NTAb bound during a 6 hour infusion.

In vitro studies have shown that close apposition of antibody molecules bound to a cell surface is necessary for engaging PMN and monocytes (Kurlander, 1978) and that in these circumstances the Fc piece continues to be active for some days after antibody binding. There are no comparable data in vivo but these heterologous experiments suggest that the Fc piece remains capable of engaging cells for a very short time after binding in vivo. A less likely alternative explanation may be that bolus injection results in non-uniform deposition of NTAb which itself is the cause of increased proteinuria.

The second observation in the autologous phase experiments, which has already been referred to, is that initial low titres of anti-sheep IgG antibody appear to protect from the harmful effects of subsequent high titres. The in vitro studies, done to determine the accessibility of sheep IgG fixed in the kidney to rabbit anti-sheep IgG, have shown that binding sites available shortly after

injection of NTG, are saturated by 21 days and probably much sooner. Further studies are required to define just how rapidly this saturation takes place.

The implications of these observations for anti-GBM antibody mediated disease (for example, Goodpasture's syndrome) in humans are considerable. After recovery from Goodpasture's syndrome, some patients continue to have high titres of anti-GBM antibody in the circulation for many months without overt evidence of disease activity (Rees, 1978; Bailey, 1981; Simpson, 1982;). It seems likely that saturation of antigenic sites on their GBM, such as occurred in the autologous phase experiments, limits further antibody deposition. Under such circumstances, fresh injury could result either from a sudden increase in the affinity of anti-GBM antibody produced, which would displace previously fixed antibody of lower affinity, or by non-specific enhancement of injury such as occurs with intercurrent bacterial infection (Rees, Lockwood & Peters 1977).

Alternatively, new antigenic sites on the GBM may become exposed as the result of direct damage. This may occur due to the deposition of immune complexes (as could occur with intercurrent infections), or as in the case of the lung, by direct damage due to the inhalation of volatile hydrocarbons or possibly pulmonary infections. These events are well described as precipitating causes of anti-GBM

antibody mediated diseases.

A better understanding of all the mechanisms involved, not only of the causation, but also of the factors responsible for relapse and progression of allergic renal disease, will hopefully lead to more effective prevention and treatment of this disorder than is currently possible.

APPENDIX

ABBREVIATIONS

Ab	antibody
Ag	antigen
BM	basement membrane
BSA	bovine serum albumin
Ci	curie
C_3, 1q...	complement components
CPS	C-polysaccharide
CRP	C-reactive protein
CVF	cobra venom factor
EDTA	ethylenediaminetetraacetate sodium
FA	Freund's adjuvant (incomplete)
FCA	Freund's complete adjuvant
FITC	fluoroisothiocyanate
GBM	glomerular basement membrane
H & E	haematoxylin and eosin
HLA	histocompatibility antigens
IV	intravenous
mci	millicurie
MHC	major histocompatibility complex
MPS	mononuclear phagocyte system
Nef	nephritic factor
NHS	normal human serum
NTAb	nephrotoxic antibody
NTG	nephrotoxic globulin
NTN	nephrotoxic nephritis
NTS	nephrotoxic serum
NZB	New Zealand brown
NZW	New Zealand white
PBS	phosphate buffered saline
PEG	polyethylene glycol
PMN	polymorph nuclear leucocyte
RSS	rabbit serum saline
SAA	serum amyloid A-protein
SLE	systemic lupus erythematosus
TBM	tubular basement membrane
TCA	trichloracetic acid
uCi	microcurie
VBS	veronal buffered saline

METHODS (EXTRA)

(i) PREPARATION OF RABBIT GLOMERULAR BASEMENT MEMBRANE GBM

Method of Krakower and Greenspon (1951) as modified by Spiro (1967) and Westberg and Michael (1970). This method of purification of rabbit GBM was used by Dr. M. Thomson to prepare the GBM which I used to raise anti-rabbit GBM antibodies in sheep.

(ii) PURIFICATION OF CRP BY AFFINITY CHROMATOGRAPHY

Method of Osmond (1975) used by Dr. M. Pepys to prepare highly purified CRP used in my later experiments.

(i) PREPARATION OF RABBIT GLOMERULAR BASEMENT MEMBRANE

(a) Isolation of glomeruli

Thirty kidneys were removed from normal rabbits, stripped of their capsule and fat and stored at -40°C. They were then almost completely thawed in a plastic bag immersed in water (22-24°C). The medulla was dissected from the cortex and discarded whilst the cortex was then minced in a household meat mincer.

Minced cortex was transferred to a 100-mesh (pore size 150 microns) stainless steel sieve (Endecotts Test Sieves Ltd.), and forced through the sieve holes with the bottom of a glass beaker. The sieve was repeatedly brushed with a wire brush and washed with cold 0.85% sodium chloride solution to clear obstructed pores. Material was forced through until only pale tissue (connective tissue and medulla) was left on the sieve. The sieved suspension was then poured through a 60-mesh sieve (pore size 250 microns) which retained larger tissue fragments such as tubules that had passed through the 100-mesh sieve end on. The sieved suspension was finally passed through a 250-mesh sieve (pore size 63 microns). The material retained by this latter sieve contained the glomeruli, which were repeatedly washed with cold 0.85% sodium chloride until nothing but glomeruli were seen on phase contrast microscopy. The glomeruli were washed from the sieve into a beaker, with streams of cold 0.85% sodium chloride and then transferred

to 50 ml plastic centrifuge tubes. The suspension was centrifuged at 1 500 r.p.m. for 10 minutes. The sediment was washed four times in distilled water with centrifugations at 2800 r.p.m. for 10 minutes and then examined by phase contrast microscopy to confirm the purity of the glomeruli.

(b) Preparation of particulate GBM

The glomerular sediment was suspended in ice-cold 1 M sodium chloride using a volume twenty times that of the sediment. The glomeruli were sonicated in 30 ml portions in 50 ml beakers immersed in an ice bath, using an MSE Ultrasonicator Disintegrator Model 150 W, with a 9 mm stainless steel probe. Bursts of sonication of 30 seconds were given with 1-minute periods of cooling of the probe in ice water between bursts. After a total sonication time of 10 minutes, and allowing several minutes for the glomeruli to settle, the suspension was examined under the phase contrast microscope to see if all glomeruli had been disrupted. Sonication was repeated until this had been achieved.

The suspension was finally passed through a 250-mesh sieve to remove non-disrupted glomeruli and tubule fragments. The filtrate was transferred to 50 ml tubes and centrifuged at 3 000 r.p.m. for 15 minutes. The sediment was then suspended in 1 M sodium chloride washed, centrifuged three

times and then suspended in 50 ml of distilled water, washed and centrifuged four times at 3000 r.p.m. for 5 minutes. The sediment was finally suspended in 4 ml of distilled water, lyophilysed and stored at -70°C until it was used as particulate GBM, or was solubilised with collagenase.

(c) Preparation of soluble GBM

Particulate GBM was solubilised by proteolytic enzyme (Collagenase-Sigma Chemical Company, St Louis, U.S.A.) by the method of Spiro (1967b) with the modification that the incubation with the enzyme was prolonged to 5 days and not 3. The digestion was performed in 0.01 M Tris acetate-buffer (pH 7.4), in the presence of 0.005 M calcium acetate at 37°C. The particulate GBM was suspended in Tris buffer (25 mg/ml). Collagenase was added initially in a quantity of 0.7 mg per 100 mg of GBM. At 24, 48, 72 and 96 hours, further enzyme in quantities of 35 mg, 10 mg, 10 mg, 10 mg per 100 mg of GBM respectively was added. A crystal of thymol was added at the beginning of the incubation to prevent bacterial growth. During the period of incubation the mixture was constantly stirred. On the sixth day the suspension was centrifuged at 2 000 r.p.m. for 30 minutes. The supernatant containing the soluble GBM was removed, heated at 60°C for 30 minutes and stored at -20°C until used. The concentration of soluble GBM in the supernatant was determined by the Folin phenol reagent (Lowry 1951).

(ii) PURIFICATION OF CRP

Pneumococcal C polysaccharide was isolated from culture of a Cs capsulated pneumococcal variant as described by Gotschlich and Liu (1967).

It was coupled to commercially available Sepharose 4B-CNBr beads according to the manufacturers' instructions (Pharmacacia (GB) Ltd., London).

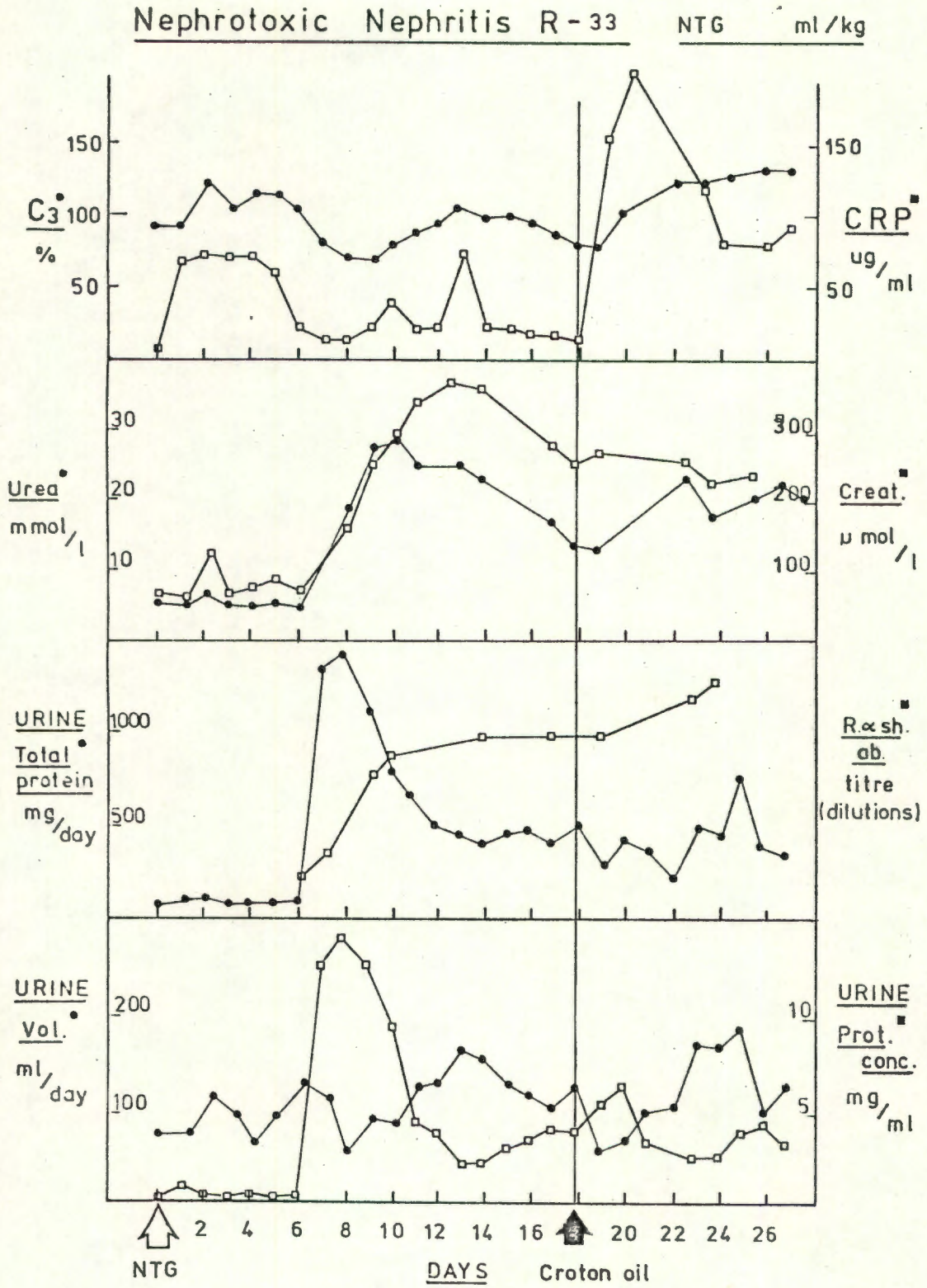
Isolation by calcium-dependant affinity chromatography

The solid phase ligand was equilibrated in a column with Tris-buffered isotonic saline containing 1 mM calcium before being allowed to react with CRP-rich rabbit serum at room temperature. The column was then washed extensively with the Tris buffer until the column effluent was free of material absorbing at 280 nm.

CRP was then eluted with Tris buffered saline, pH 8.0 containing 0.05 M sodium citrate, and the concentration of the protein assessed by the Folin method. The purity of the preparation was assessed by gel diffusion and electro-immunodiffusion.

NTN CASE RECORDS

Four case records illustrating events occuring during NTN are included. They show rabbits developing autologous phase injury with varying grades of severity. Records of this kind were kept on all rabbits for the duration of the experiments.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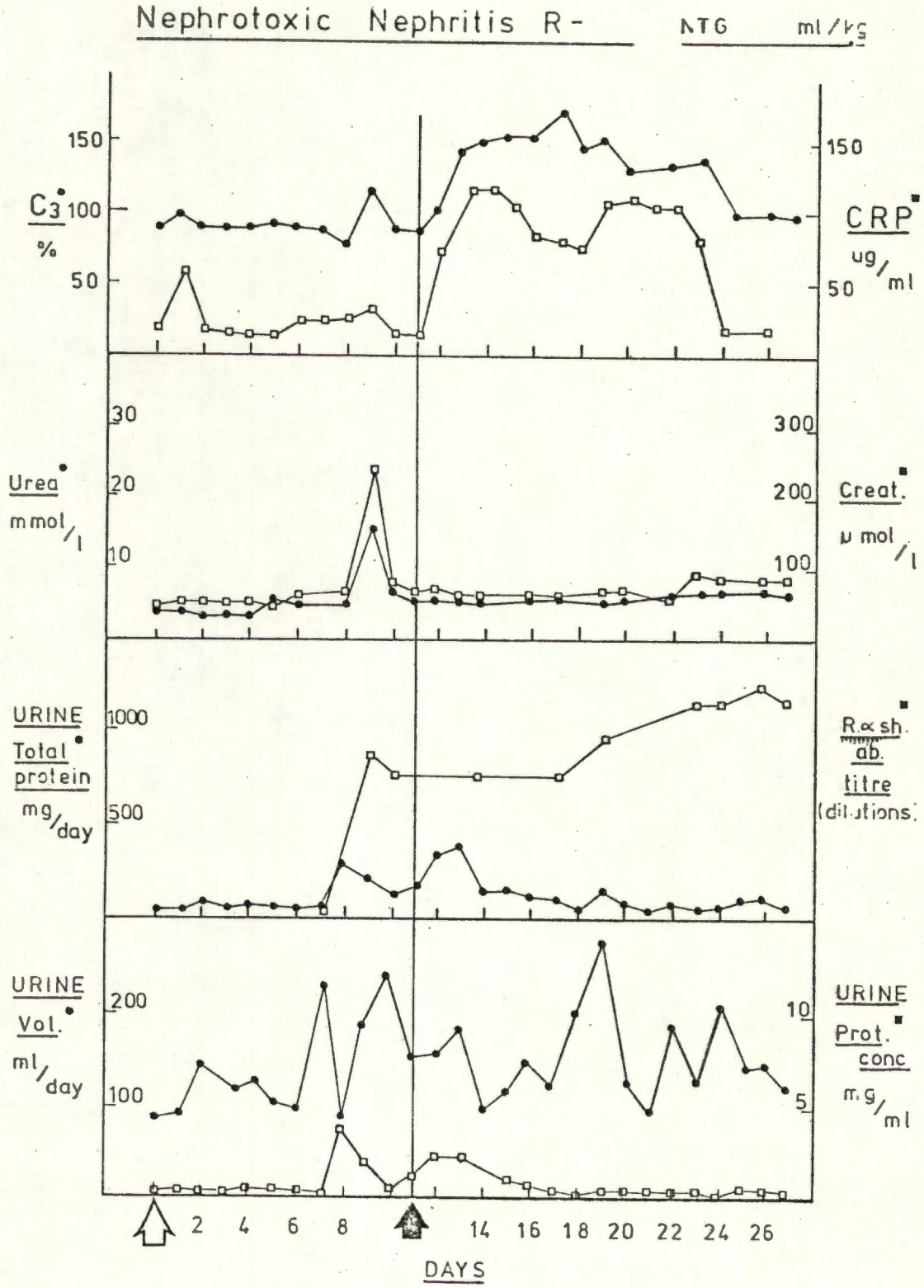
NTG ml/kg
C3 %
150
100
50
CRP ug/ml
150
50
Urea mmol/l
30
20
10
Creat. µ mol/l
300
200
100
URINE Total protein mg/day
1000
500
R.∝sh. ab. titre (dilutions)
URINE Vol. ml/day
200
100
URINE Prot. conc. mg/ml
10
5
2 4 6 8 10 12 14 16 18 20 22 24 26
NTG
DAYS
Croton oil


Nephrotoxic Nephritis R-
NTG ml/kg
C3 %
150
100
50
CRP ug/ml
150
50
Urea mmol/l
30
20
10
Creat. µ mol/l
300
200
100
URINE Total protein mg/day
1000
500
R.α sh. ab. titre (dilutions)
URINE Vol. ml/day
200
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URINE Prot. conc mg/ml
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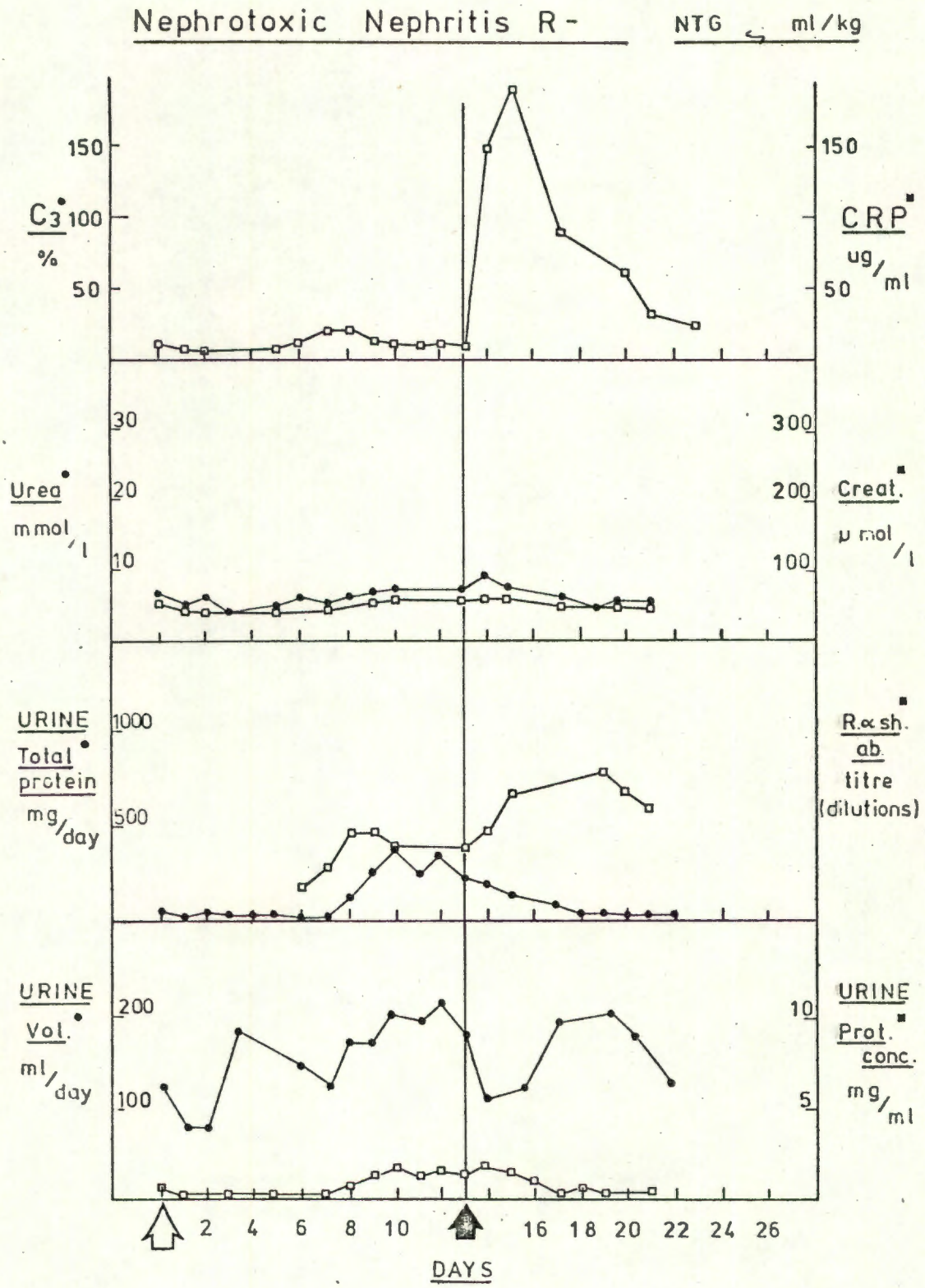
2 4 6 8 14 16 18 20 22 24 26
DAYS


Nephrotoxic Nephritis R-
NTG ml/kg
C3 %
150
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CRP ug/ml
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Urea mmol/l
30
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10
Creat. µ mol/l
300
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100
URINE Total protein mg/day
1000
500
R.α sh. ab. titre (dilutions)
URINE Vol. ml/day
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10
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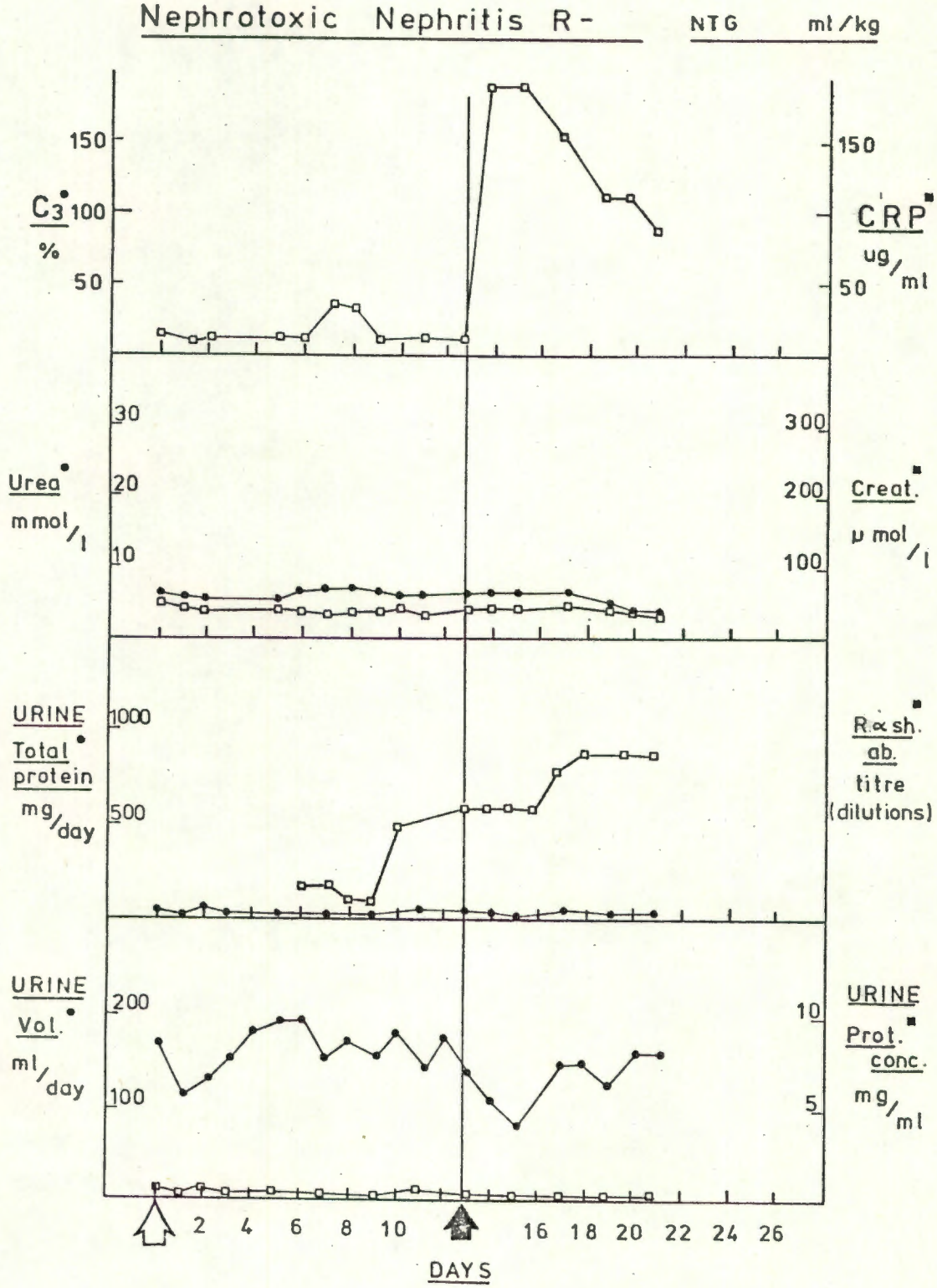
DAYS


Nephrotoxic Nephritis R-
NTG ml/kg
150
C3 100
%
50
150
CRP
ug/ml
50
30
20
10
Urea
mmol/l
300
200
100
Creat.
μ mol/l
URINE
Total protein
mg/day
1000
500
R.sh. ab. titre (dilutions)
URINE
Vol.
ml/day
200
100
10
5
URINE
Prot. conc.
mg/ml
2 4 6 8 10 16 18 20 22 24 26
DAYS

REFERENCES

REFERENCES

Abernethy,T.J. and Avery,O.T. 1941
The occurence during acute infections of a protein not normally present in the blood.I. Distribution of the reactive protein in patients' sera and the effect of calcium on the flocculation reaction with C-polysaccharide of pneumococcus. J. Exp. Med. 73:173

Abernethy,T.J. and Francis,T. 1937
Studies on the somatic C-polysaccharide of pneumococcus I. Cutaneous and serological reactions in pneumonia. J. Exp. Med. 65:59

Adler,S., Wang.H., Cohen,A. and Border,W.A. 1981
Electropharmacologic modulation of in situ complex formation in experimental membranous nephropathy. American Society of Nephrology, Washington. Abstract.

Agnello,V. 1978
Complement deficiency states. Medicine. 57:1

Alexander,J.W., Ogle,C.K., Stinnet,J.D. and MacMillan.B.G. 1978
A sequential prospective analysis of immunologic abnormalities and infection following severe thermal injury. Ann. Surg. 188: 809.

Alfiler,C.A., Roy,L.P., Doran,T., Sheldon,A. and Bashir,H. 1980
HLA-DRW 7 and steroid-responsive nephrotic syndrome of childhood. Clin. Nephrol. 14:71

Amos, R.S., Constable, T.J., Crockson, R.A., Crockson, A.P. and McConkey, B.D. 1977
Rheumatoid arthritis: relation of serum C-reactive protein and erythrocyte sedimentation rates to radiographic changes. Br. Med. J. 1:195

Atkins, R.C., Holdworth, S.R., Glasgow, E.F. and Mathews, F.E. 1976
The macrophage in human rapidly progressive glomerulonephritis. Lancet 1:830

Atkinson, J.P. and Frank, M.M. 1974
Studies on the in vivo effect of antibody. Interaction of IgM antibody and complement in the immune clearance and destruction of erythrocytes in man. J. Clin. Invest. 54:339

Avrameas, S. 1969
Coupling of enzymes to proteins with glutaraldehyde: use of the conjugates for the detection of antigens and antibodies. Immunochemistry 6:43

Avrameas, S., Tandon, B. and Chuilon, S. 1969
Glutaraldehyde, cyanuric chloride and tetra-azotized O-dianisidine as coupling reagents in the passive haemagglutination test. Immunochemistry 6:67

Baehr, G. 1926
A benign and curable form of haemorrhagic nephritis. J.A.M.A. 86:1001

Bailey, R.R., Simpson,I.J., Lyn, K.L.,Neal, T.J., Doak, P.B. and McGiven, A.R. 1981
Goodpasture's syndrome with normal renal function. Clin. Nephrol. 15:211

Ballow, M. and Cochrane, C.G. 1969
Two anticomplementary factors in cobra venom: Haemolysis of guinea-pig erythrocytes by one of them. J. Immunol. 103:944

Baltz, M.L., Gomer, K., Davies, A.J.S., Evans, D.J., Klauss, G.G.B. and Pepys, M.B. 1980
Differences in the acute phase responses of serum amyloid P-component (SAP) and C3 to injections of casein or bovine serum albumin in amyloid-susceptible and -resistant mouse strains. Clin. Exp. Immunol. 39:355

Barabas, A.Z. and Lannigan, R. 1974
Induction of an autologous immune-complex glomerulonephritis in the rat by intravenous injection of heterologous anti-rat kidney tubular antibody: I. Production of chronic progressive immune-complex glomerulonephritis. B.J. Exp. Path. 55:47

Bartlow, B.G. 1979
New immunoglobulin activating factor in membranoproliferative glomerulonephritis. Kidney Int.15:294

Bartolotti, S.R. and Peters, D.K. 1978
Delayed removal of renal-bound antigen in decomplemented rabbits with acute serum sickness. Clin. Exp. Immunol. 32:199

Beaufils, M., Morel-Maroger, L. and Sraer, J.D. 1978
Acute renal failure of glomerular origin during visceral abcesses. N. Eng. J. Med. 295:85

Becker, E.L., Showell, H.J. and Henson, P.M. 1974
The ability of chemotactic factors to induce lysosomal enzyme release. I. The characteristics of the release, the importance of surfaces and the relation of enzyme release to chemotactic responsiveness. J. Immunol. 112:2047

Becker, G.J., Waldburger, M., Hughes, G.R.V. and Pepys, M.B. 1980
Value of serum C-reactive protein measurement in the investigation of fever in systemic lupus erythematosus. Ann. Rheum. Dis. 39:50

Beirne, G.J., Wagnild, J.P., Zimmerman, S.W., Macken, P.D. and Burkholder, P.M. 1977
Idiopathic crescentic glomerulonephritis. Medicine 56:349

Beisel, W.R. 1977
Magnitude of the host nutritional responses to infection. Am. J. Clinical Nutrition 30:1236

Benacerraf, B. 1981
Role of MHC gene products in immune regulation. Science 212:1229

Benacerraf, B. and McDevitt, H.O. 1972
Histocompatibility-linked immune response genes. Science 195:273

Bennett, C.M., Glassock, R.J., Chang, R.L.S., Deen, W.M., Robertson, C.R. and Brenner, B.M. 1976
Perm-selectivity of the glomerular capillary wall: studies of experimental glomerulonephritis in the rat using dextran sulphate. J. Clin. Invest. 57:1287

Benveniste, J., Henson, P.M. and Cochrane, C.G. 1972
Leucocyte dependent histamine release from rabbit platelets. J. Exp. Med. 136:1356

Bernheim, H.A., Block, L.H. and Atkins, E. 1979
Fever: Pathogenesis, pathophysiology and purpose. Ann. Int. Medicine 91:261

Bernstein, D.L. and Walsh, E.C. 1978
Endogenous mediators of the acute phase reaction. 1. Rabbit granulocytic pyrogen and its chromatographic subfractions. J. Lab. Clin. Medicine 9:236

Bhan, A.K., Schneeberger, E.E., Collins, A.B. and McCluskey, R.T. 1978
Evidence for a pathogenic role of a cell mediated immune mechanism in experimental glomerulonephritis. J. Exp. Med. 148:246

Blackburn, G.L. 1977
Lipid metabolism in infection. Am. J. Clin. Nutrition 30:1321

Blantz, R.C. and Wilson, C.B. 1976
Acute effects of antiglomerular basement membrane antibody on the process of glomerular filtration in the rat. J. Clin. Invest. 58:899

Bohrer, M.P., Baylis, C., Humes, H.D., Glassoch, R.J., Robertson, C.R. and Brenner, B.M. 1978
Perm selectivity of the glomerular capillary wall: Facilitated filtration of circulating polycations. J. Clin. Invest. 61:72

Border, W.A. 1979
Immune complex detection in glomerular diseases. Nephron 24:105

Border, W.A., Ward, H.J., Kamil, E.S. and Cohen, A.A. 1982
Inudction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen: demonstration of a pathogenic role for electrical charge. J. Clin. Invest. 69:451

Brenner, B.M., Hostetter, T.H. and Humes, H.D. 1978
Molecular basis of proteinuria of glomerular origin. N. Eng. J. Med. 298:826

Briggs, J.D., Timbury, M.C., Paton, A.M. and Bell, P.R.F. 1972
Viral infections and renal transplant rejection. Br. Med. J. 5:520

Byrd, L.H., Tapia, L., Cheigh, J.S., Aronian, J., Stenzel, K.H. and Rubin, A.L. 1978
Association between Streptococcus faecalis urinary infections and graft rejection in kidney transplantation. Lancet 2:1167

Cameron, J.S. 1979
The nephritis of Schoenlein-Henoch Purpura: Current problems. In Progress in Glomerulonephritis. Eds. P Kincaid Smith, A.J.F. d'Apice, R.C. Atkins. John Wiley and Sons Inc. 283

Cameron, J.S., Turner, D.R., Ogg, C.S., Sharpstone, P. and Brown, C.B. 1974
The nephrotic syndrome in adults with "minimal change" glomerular lesions. Q. J. Med. 43:461

Cameron, J.S., Peters, D.K. and Rees, A.J. 1982
In Recent Advances in Renal Medicine. Eds. N.F. Jones and D.K. Peters. Churchill-Livingstone.

Cairns, S.A. 1980
Role of the major histocompatibility system in idiopathic membranous nephropathy: Prediction of outcome and response to treatment. Kidney Int. 18:802

Cattell, V. and Jamieson, S.W. 1978
The origin of glomerular crescents in experimental nephrotoxic serum nephritis in the rabbit. Lab. Invest. 39:584

Chatenoud, L. and Bach, M.A. 1981
Abnormalities of T cell subsets in glomerulonephritis and systemic lupus erythematosus. Kidney Int. 20:267

Clark, W.F., Tevaarwerk, G.J.M., Bandali, K., Lindsay, R.M. and Linton, A.L. 1980
Platelet aggregation and release associated with immune complex formation in pig plasma. J. Lab. Clin. Med. 96:654

Clarkson, A.R., Seymour, A.E., Thompson, A.J., Chan,Y.L. and Woodroffe, A.J. 1977
IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. Clin. Nephrol. 8:459

Claus, D.R., Osmond, A.P. and Gewurz, H. 1976
Radio-immuno assay of human CRP and levels in normal sera. J. Lab. Clin. Med. 87:120

Cochrane, C.G. 1971
Mechanisms involved in the deposition of immune complexes in tissues. J. Exp. Med. 134:75

Cochrane,C.G. 1979
Mediator systems in neutrophil-independent immunologic injury of the glomerulus. In: Contemporary issues in Nephrology. Eds. C.B.Wilson, B.M.Brenner and J.H.Stein. p.106 Churchill Livingstone, New York.

Cochrane, C.G. and Hawkins, D. 1968
Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. J. Exp. Med. 127:137

Cochrane, C.G. and Koffler, D. 1973
Immune complex disease in experimental animals and man. Advances in Immunology. 16:185

Cochrane, C.G., Muller-Eberhard, H.J. and Aikin, B.S. 1970
Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. J. Immunol. 105:55

Cochrane, C.G., Unanue, E.R. and Dixon, F.J. 1965
A role of polymorphonuclear leucocytes and complement in nephrotoxic nephritis. J. Exp. Med. 122:99

Cohn, Z.A. and Morse, S.I. 1960
Interactions between rabbit polymorphonuclear leucocytes and staphylococci. J. Exp. Med. 110:419

Costanza, M.E., Pinn, V., Schwartz, R.S. and Nathanson, L. 1973
Carcino-embryonic antigen-antibody complexes in a patient with colonic carcinoma and nephrotic syndrome. N. Engl. J. Med. 289:520

Cotran, R.S. 1981
The role of monocytes and macrophages in glomerulonephritis. In: Proceedings of the 8th International Congress of Nephrology, Athens, June 1981 (Introduction), Basel 1981, S. Karger, P. 853

Couser, W.G. and Salant, D.J. 1980
In situ immune complex formation and glomerular injury. Kidney Int. 17:1

Couser, W.G., Steinmuller, D.R., Stilmant, M.M., Salant, D.J. and Lowenstein, L.M. 1978
Experimental glomerulonephritis in the isolated perfused rat kidney. J. Clin. Invest. 72:1275

Couser, W.G.,, Stilmant, M.M. and Darby, C. 1976
Autologous immune complex nephropathy I. Sequential study of immune complex deposition, ultra-structural changes, proteinuria and alterations in glomerular sialoprotein. Lab. Invest. 34:23

Couser, G.C., Stilmant, M.M. and Jermanovich, N.B. 1977
Complement-independent nephrotoxic nephritis in the guinea-pig. Kidney Int. 11:170

Couser, W.G., Wagonfield, J.B., Spargo, B.H. and Lewis, E. 1974
Glomerular deposition of tumour antigen in membranous nephropathy associated with colonic carcinoma. Am. J. Med. 57:962

Crofton, R.W., Dieselhoff-Den Duck, M.M.C. and Van Furth,R. 1978
The origin, kinetics and characteristics of the kupfer cells in the normal steady state. J. Exp. Med. 148:1

Czop, J. and Nussensweig, V. 1976
Studies on the mechanism of solubilization of immune precipitates by serum. J. Exp. Med. 143:615

Daniels, C.A., Borsos, T., Rapp, H.J., Snyderman, R. and Notkins, A.L. 1969
Neutralization of sensitized virus by purified components of complement. Proc. Nat. Acad. Sci. 65:528

Daniels, J.C., Larson, D.L., Abston, S. and Ritzmann, S.E. 1974
Serum protein profiles in thermal burns. II. Protease inhibitors, complement factors and C-reactive protein. J. Trauma 14, No. 2:153

David, D.S., Millian, S.J. and Whitsell, J.C. 1972
Viral syndromes and renal homograft rejection. Ann. Surg. 175:257

Davies, M., Coles, G.A. and Hughes, K.T. 1980
Glomerular basement membrane injury by neutrophil and monocyte neutral proteinases. Renal Physiol. 3:106

Day, N.K., Geiger, M,and McLean, R. 1973
The asssociation of respiratory infection , recurrent haematuria and focal glomerulonephritis with activation of the complement system in the cold. J. Clin. Invest. 52:1968

Devey, M.E. and Steward, M.W. 1980
The induction of chronic antigen-antibody complex disease in selectively bred mice producing either high or low affinity antibody to protein antigens. Immunology 41:303

Deuel, T.F., Senior, R.M., Chang, D., Griffin, G.L., Henrikson, R.L. and Kaiser, E.T. 1981
Platelet factor 4 is chemostatic for neutrophils and monocytes. Proc. Nat. Acad. Sci., U.S.A. 78:4584

Dinarello, C.A. and Wolf, S.M. 1978
Pathogenesis of fever in man. New Engl.J. Med. 298:607

Dixon, F.J. 1970
What are sensitized cells doing in glomerulonephritis? N. Engl. J. Med. 283:536

Dixon, F.J., Feldman, J.D. and Vaquez, J.J. 1961
Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J. Exp. Med.113:899

Dossetor, J.B., Schlaut, J., Olson, L.A. and Zabriskie, J.B. 1978
Streptococcal infection as a cause of hyperacute renal allograft rejection. Transplant Proc. 10:483

Douglas, M.F.S., Rabideau, D.P., Schwartz, M.M. and Lewis, E.J. 1981
Evidence of autologous immune complex nephritis. New Engl. J. Med. 305:1326

Druet, E., Sapin, C., Gunther, E., and Druet, P. 1977
Mercuric chloride-induced anti-glomerular basement membrane antibodies in the rat: genetic control. Eur.J.Immunol. 7:348

Dubois, E.L. 1974
Lupus erythematosus. 2nd ed. Los Angeles: University of South California Press

Duffus, P., Parbtani, A., and Cameron, J.S. 1982
Intraglomerular localization of platelet related antigens, platelet factor 4 and B-thromboglobulin in glomerulonephritis. Clin. Nephrol. 17:288

Egido, J., Sanchez-Crespo, M., Lahoz, C., Garcia, R., Lopez-Trascasa, M. and Hernando, L. 1980
Evidence of an immediate hypersensitivity mechanism in systemic lupus erythematosus. Ann. Rheumat. Dis.39:312

Elin, R.L., Wolff, S.M.and Finch, C.A. 1977
Effect of induced fever on serum iron and ferritin concentrations in man. Blood 49:147

Fahey, J.L. and Terry, E.W. 1973
Ion exchange chromatography and gel filtration. In "Handbook of experimental immunology". Ed. Weir, P.M. 2nd ed. Blackwell Scientific Publications, Oxford.

Fauchet, R., Guegen, M., Genetet, B., Le Pogamp, P., Ramee, M.P., Chevet, D. and Simon, P. 1980
HLA DR antigen and IgA nephropathy (Berger's disease). N. Engl. J. Med. 302:1033

Fiedel, B.A., Simpson. R.M. and Gewurz, H. 1982
Effects of C-reactive protein (CRP) on platelet function. Ann. N.Y. Acad. Sci. 389:263

Finkelstein, F.O., Siegel, N.J., Bastl, C., Forrest, J.N.and Kashgarian, M. 1976
Kidney transplant biopsies in the diagnosis and management of acute rejection reactions. Kidney Int. 10:171

Fish, A.J., Carmody, K.M. and Michael, A.F. 1979
Distribution of antigens of human glomerular basement membrane. J. Lab. Clin. Med. 94:447

Fleuren, G., Grond, J. and Hoedemaeker, P.J. 1980
In situ formation of subepithelial glomerular immune complexes in passive serum sickness. Kidney Int. 17:631

Frank, M.M., Hamburger, M.I., Lawley, T.J., Kimberley, R.P. and Plotz, P.H. 1979
Defective reticulo-endothelial system Fc-receptor function in systemic lupus erythematosus. New Engl. J. Med. 300:518

Friend, P.S., Kim, Y., Michael, A.F. and Donadio, J.V. 1977
Pathogenesis of membranous nephropathy in systemic lupus erythematosus: possible role of non-precipitating DNA antibody. B. Med. J. 1:25

Friend, P.S., Yunis, E.J., Noreen, H.J. and Michael, A.F. 1977
B-cell alloantigen associated with chronic mesangio-capillary glomerulonephritis. Lancet 1:562

Gallagher, J.S. and Voss, E.W. 1974
Conformational state of chicken 75 immunoglobulin. Immunochem. 11:461

Ganrot, P.O. and Kindmark, C.O. 1969
A simple two-step procedure for isolation of C-reactive protein. Biochim. Biophys. Acta. 194:443

Gatti, R.A. 1971
Serum inhibitors of lymphocyte responses. Lancet 1:1351

George, C.R.P., Slichter, S.J., Quadracci, G., Meca, G. and Harker, L.A. 1974
A kinetic evaluation of hemostasis in renal disease. N. Engl. J. Med. 293:1111

Germuth, F.G. Jr. and Rodriquez, E.L. 1973
Immunopathology of the renal glomerulus. Little Brown, Boston.

Gewurz, H., Mould, C., Siegel, J. and Fiedel, B.A. 1982
Advances in Interal Medicine. Year book. Ed. G.H.Stollerman 27:345 Medical Publishers Chicago.

Glass, D., Raum, D., Gibson, D., Stillman, J.S. and Schur, P.H. 1976
Inherited deficiency of the second component of complement: rheumatic disease associations. J. Clin. Invest. 58:853

Golbus, S.M. and Wilson, C.B. 1979
Experimental glomerulonephritis induced by in situ formation of immune complexes in the glomerular capillary wall. Kidney Int. 16:148

Gotschlich, E.C., Liu, T-Y. and Oliveira, E. 1982
Binding of C-reactive protein to C-carbohydrate and PC-substituted protein. Ann. N.Y. Acad. Sci. 389:163

Gupta, R.C., McDuffie, F.C., Huston, K.A., Tappeiner, G., Meurer, M. and Jordan, R.E. 1979
Comparison of three immuno-assays for immune complexes in rheumatoid arthritis. Arthritis and Rheumatism 22:433

Haakenstad, A.O. and Mannik, M. 1974
Saturation of the reticuloendothelial system with soluble immune complexes. J. Immunol. 112:1939

Haakenstad, A.O. and Mannik M. 1976
The disappearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. Lab. Invest. 35:283

Halbwachs, L., Leveille, M., Lesavre, P.H., Wattel, S. and Leibowitch, J. 1980
Nephritic factor of the classical pathway of complement: Immunoglobulin G autoantibody directed against the classical pathway C3 convertase enzyme. J. Clin. Invest. 65:1249

Hammer, D.K. and Dixon, F.J. 1963
Experimental glomerulonephritis. II. Immunologic events in the pathogenesis of nephrotoxic serum nephritis in the rat. J. Exp. Med. 117:1019

Heide, K. and Schwick, H.G. 1973
Salt fractionation of immunoglobulins. In "Handbook of experimental immunology". Ed. Weir, D.M. 2nd ed. Blackwell Scientific Publication, Oxford.

Henson, P.M. and Spiegelberg, H.L. 1973
Release of serotonin from human platelets induced by aggregated immunoglobulins of different classes and subclasses. J. Clin. Invest. 52:1282

Heusser, C., Boesman, M., Nordon, J.H. and Isliker, H. 1973
Effect of chemical and enzymatic radio-iodination on in vitro human C1q activities. J. Immunol. 110:820

Heymann, W., Hakel, D.B., Harwood, S., Wilson, S.G.F. and Hunter, J.L.P. 1959
Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. Proc. Soc. Exp. Biol. Med. 100:660

Holdsworth, S.R., Golbus, S.M. and Wilson, C.B. 1979
Characterization of collagenase solubilized human glomerular basement membrane antigens reacting with human antibodies. Kidney Int. 16:797 (abstract)

Holdsworth, S.R., Neale, J.T. and Wilson, C.B. 1981
Abrogation of macrophage dependent injury in experimental glomerulonephritis in the rabbit. Use of an anti-macrophage serum. J. Clin. Invest. 68:686

Horowitz, J., Volanakis, J.E. and Stroud, R.M. 1979
Rabbit CRP production after insulin injection. Proc. Soc. Exp. Biol. Med. 160:222

Honig, S., Gorevic, P. and Weissman, G. 1977
C-reactive protein in systemic lupus erythematosus. Arthr. Rheum. 20:1065

Hope, W., Martin, T.J., Chesterman, C.N. and Morgan, F.J. 1979
Human B-thromboglobulin inhibits PGI_2 production and binds to a specific site in bovine aortic endothelial cells. Nature 282:210

Hoyer, J.R., Elema, J.D. and Vernier, R.L. 1976
Unilateral renal disease in the rat. II. Glomerular mesangial uptake of colloidal carbon in unilateral aminonucleoside nephrosis and nephrotoxic serum nephritis. Lab. Invest. 34:250

Hurliman, J., Thorbecke, G. and Hochwald, G. 1966
The liver as the site of C-reactive protein formation. J. Exp. Med. 123:365

Hyman, L.R., Steinberg, A.D., Colvin, R.B. and Bernard, E.F. 1976
Immunopathogenesis of auto immune tubulo-interstitial nephritis II. Role of an immune response gene linked to the major histo-compatibility complex. J. Immunol. 117:1894

Iida, K., Fujita, T. and Inai, S. 1976
Complement fixing abilities of IgA myeloma proteins and their fragments: the activation of complement through the classical pathway.. Immunochemistry 13:747

Izui, S., Lambert, P.H. and Miescher, P.A. 1976
In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA: A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. J. Exp. Med. 144:428

Jaffe, C.J., Vierling, J.M., Jones, E.A., Lawley, T.J. and Frank, M.M. 1978
Receptor specific clearance in the reticulo-endothelial system in chronic liver diseases: Demonstration of defective C3b specific clearance in primary biliary cirrhosis. J. Clin. Invest. 62:1069

James, K., Baum, L.L., Vetter, M.L. and Gewurz, H. 1982
Interactions of C-reactive protein with lymphoid cells. Ann. N.Y. Acad. Sci. 389:274

Jerslid, C., Rubinstein, P. and Day, N.K. 1976
The HLA system and inherited deficiencies of the complement system. Transplantation Review 32:33

Johnson, J.P., Whitman, W., and Wilson, C.B. 1978
Plasmaphoresis and immunosuppressive agents in anti-basement membrane antibody-induced Goodpasture's syndrome. Am. J. Med. 65:354

Kaplan, M.H. and Volanakis, J.E. 1974
Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. J. Immunol. 112:2135

Katz, F.E. Steward, M.W. 1976.
Studies on the genetic control of antibody affinity: The independent control of antibody levels and affinity in Biozzi mice. J. Immunol. 117:477

Kindmark, C.O. 1971
Stimulating effect of C-reactive protein on phagocytosis of various species of pathogenic bacteria. Clin. Exp. Immunol. 8:941

Kincaid-Smith, P. 1975
Participation of intra-vascular coagulation in the pathogenesis of glomerular and vascular lesions. Kidney Int. 7:242

Kniker, W.T. and Cochrane, C.G. 1965
Pathogenetic factors in vascular lesions of experimental serum sickness. J. Exp. Med. 122:83

Kohler, P.F., Percy, J., Campion, W.M. and Smyth, C. 1976
Hereditary angio-oedema and familial lupus erythematosus in identical twin boys. Am. J. Med. 56:406

Kondo, Y., Shigematsu, H. and Kobayashi, Y. 1972
Cellular aspects of rabbit masugi nephritis. II. Progressive glomerular injuries with crescent formation. Lab. Invest. 27:620

Konijin, A.M. and Hershiko, C. 1977
Ferritin synthesis in inflammation. Pathogenesis of impaired iron release. Br. J. Haematol. 37:7

Krakkower, C.A. and Greenspon, S.A. 1951
Localization of the nephrotoxic antigen within the isolated renal glomerulus. Arch. Path. 51:629

Kreiger, A., Thoenes, G.H. and Gunther, E. 1981
Genetic control of autoimmune tubulointerstitial nephritis in rats. Clin. Immunol. and Immunopathol. 21:301

Kurlander, R.J., Rosse, W.F. and Logue, G.L. 1978
Quantitative influence of antibody and complement coating of red cells on monocyte-mediated cell lysis. J. Clin. Invest. 61:1309

Kushner, I. 1982
The phenomenon of the acute phase response. In: C-reactive protein and the plasma protein response to tissue injury. Ann. N.Y. Acad. Sci. 389:39

Kushner, I., Broder, M.L. and Karp, D. 1978
Control of the acute phase response. Serum C-reactive protein kinetics after acute myocardial infarction. J. Clin. Invest. 61:235

Kushner, I. and Feldman, G. 1978
Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. J. Exp. Med. 148:466

Kushner, I. and Kaplan M.H. 1961
Studies of acute phase protein. I. An immunohistochemical method for the locallization of Cx-reactive protein in rabbits. Association with necrosis in local inflammatory lesions. J. Exp. Med. 114:961

Kushner, I., Rakita, L. and Kaplan, M.H. 1963
Studies of acute phase protein. II. LOcallization of CxRP in heart in induced myocardial infarction in rabbits. J. Clin. Invest. 42:286

Lawley, T.J. 1980
Immune complexes and reticulo-endothelial system function in human disease. J. Invest. Dermatol. 74:339

Laurell, C.B. 1966
Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Analyt. Biochem. 15:45

Lehman, D.H. and Wilson, C.B. 1976
Role of sensitized cells in anti-tubular basement membrane interstitial nephritis. Int. Arch. Allerg. & Appl. Immunol. 51:168

Lehman, D.H., Wilson, C.B. and Dixon, F.J. 1974
Interstitial nephritis in rats immunized with heterologous tubular basement membrane. Kidney Int. 5:187

Lehman, D.H., Wilson, C.B. and Dixon, F.J. 1975
Extraglomerular immunoglobulin deposits in human nephritis. Am. J. Med. 58:765

Lentnek, A.L., Schreider, A.D. and MacGregor, R.R. 1976
The induction of augmented granulocyte adherence by inflammation. J. Clin. Invest. 57:1098

Leon, S.A., Green, A., Ehrlich, G.E., Poland, M. and Shapiro, B. 1977
Avidity of antibodies in SLE. Relation to severity of renal involvement. Arth. & Rheum. 20:23

Levy, M., Gubler, M.C. and Habib, R. 1979
New concepts in membranoproliferative glomerulonephritis. In: Progress In Nephrology. Eds. P. Kincaid-Smith, A.J.F. d'Apice, R.C. Atkins. John Wiley & Son Inc. 177

Lockwood, C.M., Amos, M. and Peters, D.K. 1979
Goodpasture's syndrome: radio-immunoassay for the measurement of circulating anti-GBM antibody. Kidney Int. 16:93 (abstract)

Lockwood, C.M., Rees, A.J., Pearson, T.A., Evans, D.J., Peters, D.K. and Wilson, C.B. 1976
Immunosuppression and plasma exchange in the treatment of Goodpasture's syndrome. Lancet 1:711

Lockwood, C.M., Worlledge, S., Nicholas, A., Cotton, C. and Peters, D.K. 1979
Reversal of impaired splenic function in patients with nephritis or vasculitis (or both) by plasma exchange. N. Engl. J. Med. 300:524

Lopes, C., Simmons, R.L., Mauer, M., Park, B., Najarian, J.S. and Good, R.A. 1973
Role of virus infections in immunosuppressed renal transplant patients. Transplant Proc. 5:803

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951
Protein measurement with the Folin phenol reagent. J. biol. Chem. 193:265

Mahieu, P., Dardenne, M. and Bach, J.F. 1972
Detection of humoral and cell mediated immunity to kidney basement membranes in human renal diseases. Am. J. Med. 53:185

Mancini, G., Carbonara, A.O. and Heremans, J.F. 1965
Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235

Mannik, M., Arend, W.P., Hall, A.P. and Gilliland, B.C. 1971
Studies on antigen-antibody complexes. I. Elimination of soluble complexes from rabbit circulation. J. Exp. Med. 133:713

Marquardt, H., Wilson, C.B. and Dixon, F.J. 1973
Isolation and immunological characterization of human glomerular basement membrane antigens. Kidney Int. 3:57

Masugi, M. 1933
Uber das wesen der spezifischen Veranderingen der Niere und der leber durch das nephrotoxin bzw. das hepatotoxin. Beitr. Pathol. Anat. Allegn. Pathol. 91:82

Mauer, S.M., Fish, A.J., Blau, E.B. and Michael, A.F. 1972
The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. J. Clin. Invest. 51:1092

Mauer, S.M., Sutherland, D.E.R., Howard, R.J., Fish, A.J., Najarian, J.S. and Michael, A.F. 1973
The glomerular mesangium. III. Acute immune mesangial injury: A new model of glomerulonephritis. J. Exp. Med. 137:553

McCluskey, R.T. and Colvin, R.B. 1978
Immunological aspects of renal tubular and interstitial diseases. Ann. Rev. Med. 29:191

McConahey, P.J. and Dixon, F.J. 1966
A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy 29:185

McCoy, R.C., Johnson, H.K., Stone, W.J. and Wilson, C.B. 1976
Variation in glomerular basement membrane antigens in hereditary nephritis. Lab. Invest. 34:325 (abstract)

McIntosh, R.M., Griswald, W.R., Chernack, W.B., Williams, G., Strauss, J., Kaufman, D.B., Koss, M.N., McIntosh, J.R., Cohen R. and Weill, R. 1975
Further studies on the nature, incidence, clinical diagnostic, prognostic and immunopathologic significance of cryoproteins in renal disease. Quarterly J. of Med. 174:285

Meadow, S.R., Glasgow, E.F., White, R.H.R., Moncrief, M.W., Cameron, J.S. and Ogg, C.S. 1972
Schoenlein-Henoch nephritis. Quarterly J. of Med. 41:242

Merriman, C.R., Pulliman, L.A. and Kampschmidt, R.F. 1975
The effect of leukocytic endogenous mediator on C-reactive protein in rabbits. Proc. Soc. Exp. Biol. Med. 149:782

Michael, A.F., Keane, W., Raij, L., Vernier, R.L. and Mauer, S.M. 1980
Editorial review of the glomerular mesangium. Kidney Int. 17:141

Miller, G.W. and Nussenzweig, V. 1975
A new complement function: solubilization of antigen / antibody aggregates. Proc. Nat. Acad. Sci. (Wash.) 72:418

Miller, K., Dresner, I.G. and Michael, A.F. 1980
Localization of platelet antigens in human kidney disease. Kidney Int. 18:472

Morimoto, C., Reinhertz, E.L., Schlossman, S.F., Schur, P.H., Mills, J.A. and Steinberg, A.D. 1980
Alterations in immunoregulatory T cell subsets in active systemic lupus erythematosus. J. Clin. Invest. 66:1171

Naish, P.F., Evans, D.J. and Peters, D.K. 1971
The effects of defibrination with ancrod in experimental allergic glomerular injury. Clin. Exp. Immunol. 20:303

Naish, P.F., Thomson, N.M., Simpson, I.J. and Peters, D.K. 1975
The role of polymorphonuclear leucocytes in the autologous phase of nephrotoxic nephritis. Clin. Exp. Immunol. 22:102

Nakashima, Y., Hirose, S. and Hamashima, Y. 1980
Proliferations of cultured rabbit renal glomerular cells stimulated by platelet factor. Acta. Pathol. Jpn. 30:1

Narkates, A.J. and Volanakis, J.E. 1982
C-reactive protein binding specificities: artificial and natural phospholipid bilayers. Ann. N.Y. Acad. Sci. 389:172

Naruse, T., Kitamura, K., Miyakawa, Y. and Shibata, S. 1973
Deposition of renal tubular epithelial antigen along glomerular capillary walls of patients with membranous glomerulonephritis. J. Immunol. 110:1163

Neale, T.J. and Wilson, C.B. 1979
Fixed glomerular antigen in Heymann's nephritis: eluted antibody reactivity with normal rat glomeruli. Kidney Int. 16:799 (abstract)

Noel, L.H., Descamps, B., Jungers, P., Bach, J.F., Busson, M., Suet, C., Hors, J. and Dausset, J. 1978
HLA antigen in three types of glomerulonephritis. Clin. Immunol. Immunopathol. 10:19

Nyulassy, S., Buc, M., Sasinka, M., Pavlovic, M., Slugen, I., Hirschova, V., Kaisenova, M., Menkyna, R. and Stefanovic, J. 1977
The HLA system in glomerulonephritis. Clin. Immunol. Immunopathol. 7:319

Oliveira, E.B., Gotschlich, E.C. and Liu, T.Y. 1979
Primary structure of human C-reactive protein. J. Biol. Chem. 254:489

Oliveira, E.B., Gotschlich, E.C. and Liu, T.Y. 1980
Comparative studies on the binding properties of human and rabbit C-reactive proteins. J. Immunol. 124:1396

O'Regan, S., Smith, M. and Drummond, K.N. 1976
Antigens in human immune complex nephritis. Clin. Nephrol. 6:417

Osmand, A.P., Friedenson, B., Gewurz, H., Painter, R.H., Hofmann, T. and Shelton, E. 1977
Characterization of C-reactive protein and the complement subcomponent Cit as homologous proteins displaying cyclic pentameric symmetry (pentraxins). Proc. Nat. Acad. Sci. U.S.A. 74: 739

Osmand, A.P., Mortensen, R.F.,and Gewurz, H. 1975
Interactions of C-reactive protein with the complement system. III. Complement dependent passive haemolysis initiated by CRP. J. Exp. Med. 142:1065

Ouchterlony, O. 1953
Antigen-antibody reactions in gels.IV. Types of reactions in coordinated systems of diffusion. Acta. Path. Microbiol. Scand. 32:231

Ouchterlony,O. 1958
Diffusion-in-gel methods for immunological analysis. In: Progress in Allergy. Ed. P. Kallos. Basel and New York: Karger. Vol. V, p. 1

Parish, W.E. 1971
Studies on vasculitis. I. Immunoglobulins, biC, C-reactive protein and bacterial antigens in cutaneous vasculitis lesions. Clin. Allergy 1:97

Parish, W.E. 1976
Studies in vasculitis. VII. C-reactive protein as a substance perpetuating chronic vasculitis. Occurrence in lesions and concentrations in sera. Clin. Allergy 6:543

Pepys, M.B. 1981
C-reactive protein fifty years on. Lancet 1:653

Pepys, M.B., Dash, A.C., Ashley, M.J. 1977
Isolation of C-reactive protein by affinity chromatography. Clin. exp. Immunol. 30:32

Pepys, M.B., Dash, A.C., Fletcher, T.C., Richardson, N., Munn, E.A. and Feinstein, A. 1978
Analogues in other mammals and in fish of human plasma proteins, C-reactive protein and amyloid P component. Nature 273:168

Pepys, M.B., Rogers, S.L. and Evans, D.J. 1982
Role of the acute phase response in the Schwartzman phenomenon. Clin. Exp. Immunol. 47:289

Pincus, T., Haberkern, R. and Christian, C.L. 1968
Experimental chronic glomerulitis. J. Exp. Med. 127:819

Pinching, A.J., Rees, A.J., Pussell, B.A., Lockwood, C.M., Mitchison, R.S. and Peters, D.K. 1980
Relapses in Wegener's granulomatosus: the role of infection. B. Med. J. 281:836

Polverini, P.J., Cotran, R.S., Gimbrone, M.A. Jr. and Unanue, E.F. 1977
Activated macrophages induce vascular proliferation. Nature, (London) 269:804

Pressman, D., Eisen, H.M. and Fitzgerald, P.J. 1950
The zone of localization of antibodies. VI. The rate of localization of anti mouse-kidney serum. J. Immunol. 64:281

Raij, L., Keane, W.F., Osswald, H. and Michael, A. 1979
Mesangial function in ureteral obstruction in the rat - blockade of the efferent limb. J. Clin. Invest. 64:1204

Rashid, H.U., Biswas, C.K., Morley, A.R. and Kerr, D.N.S. 1981
Mesangio-capillary glomerulonephritis type I associated with immunoglobulin deficiency. Br. Med. J. 283:407

Raveche, E.S., Steinberg, A.D., Klassen, L.W. and Tjio, J.H. 1978
Genetic studies in NZB mice. I. Spontaneous auto antibody production. J. Exp. Med. 147:1487

Rees, A.J., Amos, M. and Peters, D.K. 1981
Infection and heterologous phase proteinuria in nephrotoxic nephritis. Kidney Int. 20:687

Rees, A.J., Lockwood, C.M. and Peters, D.K. 1977
Enhanced allergic tissue injury in Goodpasture's syndrome by intercurrent bacterial infection. Br. Med. J. 2:723

Rees, A.J., Peters, D.K., Compston, D.A.S. and Batchelor, J.R. 1978
Strong association between HLA DRWZ and antibody mediated Goodpasture's syndrome. Lancet 1:966

Rees, A.J., Peters, D.K., Welsh, K.I., Batchelor, J.R. 1978
Genetic factors influencing the severity of antibody mediated Goodpasture's syndrome. Abstracts of 8th International Congress of Nephrology. Athens. 1981

Reinertsen, J.L., Klippel, J.H., Johnson, A.H., Steinberg, A.D., Decker, J.L. and Mann, D.L. 1978
B-lymphocyte alloantigens associated with systemic lupus erythematosus. N. Engl. J. Med. 299:515

Rocklin, R.E., Lewis, E.J. and David, J.R. 1970
In vitro evidence for cellular hypersensitivity to glomerular basement membrane antigens in human glomerulonephritis. N. Engl. J. Med. 283:497

Rosenthal, A.S. 1980
Regulation of the immune response - role of the macrophage. N. Engl. J. Med. 303:1153

Ryan, G.B., Hein, J.H. and Karnovsky, M.J. 1976
Glomerular permeability to proteins: effects of haemodynamic factors on the distribution of endogenous immunoglobulin and exogenous catalase in the rat glomerulus. Lab. Invest. 34:415

Saba, T.M. and Jaffe, E. 1980
Plasma fibronectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. Am. J. Med. 68:577

Salant, D.J., Belok, S., Stilmant, M.M. and Couser, W.G. 1978
A new role for complement in experimental glomerulonephritis. Clin. Research 26:324A

Sapin, C., Mandet, G., Druet, E., Gunther, G. and Druet, P. 1981
Immune complex type disease induced by $HgCl_2$: genetic control of susceptibility. Transplantation Proc. 13:1404

Scheidemandel, 1913
Munchen med. Wehnschr. 60:1778

Scheinman, J.I., Fish, A.J. and Michael, A.F. 1974
The immunohistopathology of glomerular antigens: the glomerular basement membrane, collagen and actinomycin antigens in normal and diseased kidneys. J. Clin. Invest. 54:1144

Scheinman, J.I., Fish, A.J., Matas, A.J. and Michael, A.F. 1978
The immunohistopathology of glomerular antigens. II. The glomerular basement membrane, actomyosin and fibroblast surface antigens in normal, diseased and transplanted human kidneys. Am. J. Pathol. 90:71

Schreiber, R.D. and Muller-Eberhard, H.J. 1979
Complement and renal disease. In: Contemporary Issues in Nephrology, vol. III. Eds. C.B. Wilson, B.M. Brenner and J.H. Stein. p. 67, Churchill Livingstone, New York.

Schreiner, G.F., Cotran, R.S., Pardo, V. and Unanue, E.R. 1978
A mononuclear cell component in experimental immunological glomerulonephritis. J. Exp. Med. 147:369

Schreiner, G.F., Cotran, R.S. and Unanue, E.R. 1981
Glomerular cell types and immune function. In: Proceedings of the 8th International Congress of Nephrology, Athens, June 1981, Basel 1981. S. Karger, p. 858

Schifferli, J.A., Morris, S.M., Dash, A. and Peters, D.K. 1981
Complement-mediated solubilization in patients with systemic lupus erythematosus nephritis or vasculitis. Clin. Exp. Immunol. 46:557

Schur, P.H. 1977
Complement testing in the diagnosis of immune and auto-immune diseases. Am. Coll. Clin. Path. 68:647

Schur, P.H. 1978
Genetics of complement deficiencies associated with lupus-like syndromes. Arthritis and Rheumatism.21:153

Schur, P.H. and Sandson, J. 1968
Immunological factors and clinical activity in systemic lupus erythematosus. N. Engl. J. Med. 278:533

Scott, D.M., Amos, N., Sissons, J.G.P., Lachmann, P.J. and Peters, D.K. 1978
The immunoglobulin nature of nephritis factor (NeF). Clin. Exp. Immunol. 32:12

Seiler, M.W., Rennke, H.G., Venkatachalam, M.A. and Cotran, R.S. 1977
Pathogenesis of polycation-induced alterations ("fusions") of glomerular epithelium. Lab. Invest. 36:48

Sharon, Z., Schwartz, M.M. and Lewis, E.J. 1977
The glomerular localization and transport of aggregated protamine-heparin complexes. Lab. Invest. 37:43

Shigematsu, H., Niwa, Y., Takizawa, J. and Akikusa, B. 1979
Arthus-type nephritis. I. Characterization of glomerular lesions induced by insoluble and poorly soluble immune complexes. Lab. Invest. 40:492

Shigematsu, H., Shishido, H., Kuhara, K. et al. 1973
Participation of monocytes in transient glomerular hypercellularity in post-streptococcal glomerulonephritis. Virchows Arch. Cell Pathol. 12:36

Sibley, W.A. and Foley, J.M. 1965
Infection and immunization in multiple sclerosis. Ann. N.Y. Acad. Sci. 122:457

Simpson, I.J., Amos, N., Evans, D.J., Thompson, N.M. and Peters, D.K. 1975
Guinea pig nephrotoxic nephritis. I. The role of complement and polymorphnuclear leucocytes and the effect of antibody subclass and fragments in the heterologous phase. Clin. Exp. Immunol. 19:499

Simpson, I.J., Doak, P.B., Williams, L.C., Blacklock, H.A., Hill, R.S., Teague, C.A., Henderson, P.B. and Wilson, P.B. 1982
Plasma exchange in Goodpasture's syndrome. Am. J. Nephrol. 2:301

Simpson, I.J., Thomson, N.M., Amos, N., Evans, D.J. and Peters, D.K. 1977
Guinea-pig nephrotoxic nephritis. II. Autologous phase: complement activation without detectable injury. Clin. Exp. Immunol. 28:146

Sindrey, M., Marshall, T.L. and Naish, P. 1979
Quantitative assessment of the effects of platelet depletion in the autologous phase of nephrotoxic nephritis. Clin. Exp. Immunol. 36:90

Sissons, J.G.P., West, R.J., Fallows, J., Williams, D.G., Boucher, B.J., Amos, N. and Peters, D.K. 1976
The complement abnormalities of lipodystrophy. N. Engl. J. Med. 294:461

Sitprija, V., Pipatanagul, V., Boonpucknavig, V. and Boonpucknavig, S. 1974
Glomerulitis in typhoid fever. Ann. Int. Med. 81:210

Snedecor, G.W. 1956
Statistical Methods. 5th ed. The Iowa State University Press, U.S.A.

Sobocinski, P.Z., Canterbury, W.J. Jr., Hauer, E.C. and Beau, F.A. 1979
Induction of hypozincemia and hepatic metallothionein synthesis in hypersensitivity reactions. Proc. Soc. Exp. Biol. Med. 160:175

Soothill, J.F. and Steward, M.W. 1971
The immunopathological significance of the heterogeneity of antibody affinity. Clin. Exp. Immunol. 9:193

Spiro, R.G. 1967a
Studies on the renal glomerular basement membrane. Preparation and chemical composition. J. Biol. Chem. 242:1915

Steblay, R.W. 1962
Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane and Freund's complete adjuvant. J. Exp. Med. 116:253

Steele, E.J., Reynolds, B.L., Rother, U. and Rowley, D. 1977
The mechanism of complement fixation by rabbit $F(ab)_2$: bactericidal activity of chelated serum and C4-deficient serum. Immunochemistry. 14:681

Stenglein, B., Thoenes, G.H. and Gunther, E. 1975
Genetically controlled autologous immune complex glomerulonephritis in rats. J. Immunol. 115:895

Steward, M.R., Reinhardt, M.C. and Staines, N.A. 1979
The genetic control of antibody affinity. Evidence from breeding studies with mice selectively bred for either high or low affinity antibody production. Immunology. 37:697

Sugisaki, T., Klassen, J., Andres, G.A., Milgrom, F. and McCluskey, R.T. 1973
Passive transfer of Heyman nephritis with serum. Kidney Int. 3:66

Sztein, M.B., Vogel, S.N., Sipe, J.D., Murphy, R.A., Mizel, S.B., Oppenheim, J.J. and Rosenstreich, D.L. 1981
The role of macrophages in acute phase response: SAA inducer is closely related to lymphocyte activating factors and endogenous pyrogen. Cell. Immunol. 63:164

Thomson, N.M. 1975
M.D. Thesis. University of Adelaide.

Thomson, N.M., Holdsworth, S.R., Glasgow, E.F. and Atkins, R.C. 1979
The macrophage in the development of experimental crescentic nephritis. Amer. J. Pathol. 94:223

Thomson, P.D., Barratt, T.M., Stokes, C.R., Turner, M.W. and Soothill, J.F. 1976
HLA antigens and atopic features in steroid responsive nephrotic syndrome of childhood. Lancet 2:765

Tillet, W.S. and Francis, T. 1930
Serologic reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J. Exp. Med. 52:561

Trevillian, P. and Cameron, J.S. 1979
The interaction of anti-glomerular basement membrane antibody deposition with immune elimination of bovine serum albumin in the rabbit. Clin. Exp. Immunol. 35:338

Trompeter, R.S., Barratt, T.M., Kay, R., Turner, M.W. and Soothill, J.T. 1980
HLA Atopy and cyclophosphamide in steroid-responsive childhood nephrotic syndrome. Kidney Int. 17:113

Turchik, J.B. and Bornstein, D.L. 1980
Role of the central nervous system in acute-phase responses to leucocytic pyrogen. Infect. Immunol. 30:439

Unanue, E.R. and Dixon, F.J. 1965
Experimental glomerulonephritis. V. Studies on the interaction of nephroxic antibodies with tissues of the rat. J. Exp. Med. 121:697

Unanue, E.F. and Dixon, F.J. 1965
Epxerimental glomerulonephritis. VI. The autologous phase of nephrotoxic serum nephritis. J. Exp. Med. 121:715

Unanue, E.R. and Dixon, F.J. 1967
Experimental glomerulonephritis. Immunologic events and pathogenetic mechanisms. Adv. Immunol. 6:1

Unanue, E.R., Schreiner, G.F. and Cotran, R.S. 1983
A role of mononuclear phagocytes in immunologically induced glomerulonephritis. In: Immune Mechanism in Renal Disease. Eds. N.B. Cummings, A.F. Michael and C.B. Wilson. New York. Plenum Pub. Corp. p. 443

Unkeless, J.C., Gordon, S. and Reich, E. 1974
Secretion of plasminogen activator by stimulated macrophages. J. Exp. Med. 139:834

Van Zyl-Smit, R. and Camacho, P. 1979
C-reactive protein in renal allograft rejection. Kidney Int. 16:91

Velosa, J.A., Miller, K. and Michael, A.F. 1976
Immunopathology of the end-stage kidney: immunoglobulin and complement component deposition in non-immune disease. Am. J. Pathol. 84:149

Velosa, J., Glasser, R.J., Nevins, T.E. and Michael, A.F. 1977
Experimental model of focal sclerosis. II. Correlation with immunopathologic changes, macromolecular kinetics and polyanion loss. Lab. Invest. 36:527

Vernier, R.L., Papermaster, B.W. and Good, R.A. 1959
Aminonucleoside nephrosis, I. Electron microscopic study of the renal lesion in rats. J. Exp. Med. 109:115

Volanakis, J.E. 1982
Complement activation by C-reactive protein complexes. Ann. N.Y. Acad. Sci. 389:235

Volanakis, J.E. and Stroud, R.M. 1972
Rabbit Ciq: Purification, functional and structural studies. J. Immunol. Methods 2:25

Wang, B.S., Heacock, E.H., Wu, A.V.O. and Mannick, J.O. 1980
Generation of suppressor cells in mice after surgical trauma. J. Clin. Invest. 66:200

Watson, J.I., Dixon, F.J. and Feldman, J.D. 1965
The effect of complete Freund's adjuvant on rat kidneys. Lab. Invest. 14:1559

Werb, Z. and Gordon, S. 1975
Elastase excretion by stimulated macrophages. Characterization and regulation. J. Exp. Med. 142:361

Westberg, N.G. and Michael, A.F. 1970
Human glomerular basement membrane. Preparation and composition. Biochemistry (Wash.) 9:3837

Whicher, J.T., Martin, M.F.R. and Dieppe, P.A. 1980
Absence of prostaglandin stimulated increase in acute phase proteins in systemic sclerosis. Lancet. 2:1187

Wilson, C.B. and Dixon, F.J. 1973
Anti-glomerular basement membrane antibody-induced glomerulonephritis. Kidney Int. 3:74

Wilson, C.B. and Dixon, F.J. 1976
The renal response to immunological injury. In: The Kidney. Eds. B.M. Brenner and F.C. Rector, Jr. p. 838, Saunders, Philadelphia.

Wilson, C.B. and Dixon, F.J. 1981
The renal response to immunological injury. In: The Kidney. Ed. B.M. Brenner and F.C. Rector. Ch. 25. Saunders, Philadelphia.

Wilson, C.B. Golbus, S.M., Neale, T.J. and Woodroffe, A.J. 1980
Nephritogenic immune responses involving antigens in or of the glomerulus. In: Streptococcal Diseases and the Immune Response. Eds. S.E. Read and Z.B. Zabrinskie. pp. 463-475, Academic Press, New York.

Wilson, C.B., Lehman, D.H., McCoy, R.C., Gunnels, J.C. Jr. and Stickel, D.L. 1974
Antitubular basement membrane antibodies after renal transplantation. Transplantation.18:467

Wilson, C.B., Marquardt, H. and Dixon, F.J. 1974
Radio-immunoassay for circulating anti-glomerular basement membrane antibodies. Kidney Int. 6:114 (abstract)

Winfield, J.B., Faiferman, I. and Koffler, D. 1977
Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity anti-native DNA antibody with glomerulonephritis. J. Clin. Invest. 59:90

Winkelhake, J.L. 1978
Immunoglobulin structure and effector functions. Immunochem. 15:695

Woo, K.T., Junor, M.J.R., Salem, H., d'Apice, A.J.F., Whitwerth, J.A. and Kincaid-Smith, P.S. 1980
Beta-thromboglobulin and platelet aggregates in glomerulonephritis. Clin. Nephrol. 14:92

Wood, B.T., Thompson, S.H. and Goldstein, G. 1965
Fluorescent antibody staining. III. Preparation of fluorescein-isothiocyanate-labelled antibodies. J. Immunol. 95:225

Wood, H.F. 1953a
The relationship between acute phase response and antibody production in the rabbit. I. Correlation between early appearance of CRP and subsequent antibody production. J. Exp. Med. 98:311

Wood, H.F. 1953b
The relationship between the acute phase response and antibody production in the rabbit. II. The stimulation of C-reactive protein response by certain adjuvants and the relation of this response to the enhancement of antibody formation. J. Exp. Med. 98:321

Zubler, R.H., Lange, G., Lambert, P.H. and Miescher, P.A. 1976
Detection of immune complexes in unheated sera by a modified ^{125}IC1q binding test. J. Immunol. 116:232